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PARTICIPANTS

Eighth Conference on Liver Injury

DR CHARLES H BEST *Chairman*
Department of Physiology University of Toronto
Toronto Canada

DR WALLACE D ARMSTRONG
Department of Physiological Chemistry University of Minnesota Medical School
Minneapolis Minnesota

DR JESSE L BOLLMAN
Department of Physiology Mayo Foundation
Rochester Minnesota

DR FRANK FREMONT SMITH
Josiah Macy Jr Foundation
New York City New York

DR HARRY GOLDBLATT
Institute for Medical Research Cedars of Lebanon Hospital
Los Angeles California

DR PAUL GYÖRGY
Department of Clinical Pediatrics University of Pennsylvania School of Medicine
Philadelphia Pennsylvania

DR FRANKLIN M HANGER
Department of Medicine Columbia University College of Physicians & Surgeons
New York New York

DR W S HARTROFT
Banting & Best Department of Medical Research University of Toronto
Toronto Canada

DR F W HOFFBAUER
Department of Medicine University of Minnesota Medical School
Minneapolis Minnesota

DR DWIGHT J INGLE
Research Department, The Upjohn Company
Kalamazoo Michigan

DR MELVIN H KNISELY
Department of Anatomy The Medical College of the State of South Carolina
Charleston South Carolina

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DR MELVIN H KNISELY
Department of Anatomy The Medical College of the State of South Carolina

DR SIDNEY C MADDEN
Division of Pathology, Brookhaven National Laboratory, Associated Universities, Inc
Upton, L I, New York

DR J. MARKOWITZ
Department of Physiology University of Toronto
Toronto, Canada

DR JOHN R NEEFE
Department of Medicine, University of Pennsylvania Hospital
Philadelphia, Pennsylvania

DR ARTHUR J PATEK, JR
Department of Medicine, Columbia University, College of Physicians & Surgeons
New York, New York

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Bethesda 14, Maryland

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New York, New York

DR DEWITT STETTEN, JR
Public Health Institute of New York
New York, New York

DR CECIL J WATSON
Department of Medicine, University of Minnesota Medical School
Minneapolis, Minnesota

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Eighth Conference On Liver Injury

SECTION I

THE LIVER LOBULE

Best I need not say that we conduct these conferences in a very informal manner The speakers may be interrupted for questions at any time We will start with Dr Knisely, who will discuss certain aspects of the liver lobule

Knisely The purpose of this paper is to present a concise view of the selective removal of coated particles from the blood stream It is necessary to understand this subject in order to understand certain aspects of those conditions and diseases in which the blood of the patient is precipitated and agglutinated into a circulating sludge

To present this subject it is necessary to describe first of all the structure and mechanical functioning of the liver lobule of frogs, and show how the phagocytic von Kupffer cells which line the sinusoids of the lobule selectively remove foreign particles from the blood stream

Methods Living internal organs have been transilluminated in situ for microscopic study by means of fused quartz rods Motion pictures of the circulation in many frog organs have been taken through the microscope, thereby recording some of the observations made In mice, rats, guinea pigs, and kittens circulatory conditions in small vessels of pia arachnoid, smooth muscles of stomach and intestinal walls, and of uteri, spleens, and livers have been studied

Problems and results The structure and mechanical functioning of the living frog liver lobule has been studied with three purposes in mind (1) to find out how the hepatic artery and portal vein terminals are distributed to and within the lobule and how their activities may effect or control the chemical environment of the hepatic parenchyma cells, (2) to find out how the lobule stores and releases blood, thereby controlling circulating blood volume and cardiac output, (3) to find out how one phagocytic von Kupffer cell selectively removes one foreign particle from the circulating blood How does the phagocyte select what it takes? Why does it ignore what it does not take? What factors determine the rates at which particles are brought to these stationary phagocytes?

The hepatic arteriole runs along the interlobular portal venule like a vine on a tree Both join the sinusoids Every sinusoid is connected to a terminal portal venule Every sinusoid, or almost every one, receives a branch the arterial sinus twig, from the hepatic artery Contractile arterio portal anastomoses interconnect the hepatic artery

and the portal venule like rungs of a ladder The sinusoids have a complete continuous cellular lining Each cell of this lining is a phagocytic von Kupffer cell In the living frog liver the von Kupffer cell is not a star shaped cell suspended by processes like a spider in a tube Each sinusoid has an afferent inlet sphincter guarding its junction with the portal vein Each sinusoid has an efferent outlet sphincter guarding its junction with the central vein The living lobules also have the small sluice channels having one common outlet to the central vein or to the sublobular vein

The degree of dilation or tonic contraction of the hepatic artery, arterio portal anastomoses, and afferent interlobular portal venule determines the relative composition of the blood which is supplied to the sinusoids, that is, of the blood which nourishes the hepatic parenchyma cells When the interlobular hepatic arteries shut off tightly throughout their lengths and the portal vein remains dilated, the sinusoids receive only pure portal vein blood When the terminal interlobular portal venules are tightly constricted and the hepatic arterioles dilated, the sinusoids receive only pure arterial blood When the hepatic artery is dilated and the arterial sinus twigs are shut off and the arterio-portal anastomoses are open, arterial blood passes into the terminals of the portal vein and is distributed by way of the portal vein tips to every sinusoid whose inlet sphincter is open With the arterio portal anastomoses closed and the portal vein terminal and hepatic artery terminals partly open the peripheral ends of the sinusoids receive a mixture of portal vein and arterial blood The kind of blood, or the ratio of the mixture, and the volume of blood supplied to a given set of sinusoids per unit of time, is continually controlled and may be maintained constant for hours at a time

These observations demonstrate beyond reasonable doubt, that the chemical environment of the hepatic parenchyma cells of each lobule is precisely controlled continuously and that it is forced to vary within controlled limits

The tubular sinusoid lining membrane which is contractile throughout its length and each cell of which is a phagocytic von Kupffer cell, has three distinct permeability phases In one the red cells enter the sinusoid separated by small volumes of plasma and the distance between the red cells does not become visibly less as the column of cells passes along the sinusoids During another extremely permeable phase the sinusoid lining membrane is probably permeable to all the colloids of the blood As the red cells pass along the sinusoids they come closer and closer together until the central two thirds, half, or third of the sinusoid contains only packed red cells moving sometimes slowly sometimes rapidly The sinusoids also exhibit an intermediate

permeability phase during which the sinusoid linings probably are permeable to proteins of small molecular weight such as albumen, but probably are not permeable to larger ones such as globulins or fibrinogen It is important to note that the experimental conditions do not cause the sinusoid linings to go into or stay in any one of their three permeability phases

The outlet sphincters of the individual sinusoids and those of Deysach s small sluice channels are the sluice mechanisms of the living frog liver Each of these sphincters can dilate widely, contract tightly, shut, or maintain any intermediate degree of tonic contraction They control the volume of blood stored in the liver and backed up in the portal vein tributaries, and thereby control the circulating blood volume and cardiac output Thus far the outlet sphincters are the only contractile structures which we have seen cause the liver to store or release blood (The anatomical distribution and mechanical behavior of the parts of the frog liver lobule s vascular system, outlined above, were demonstrated by motion pictures taken through the microscope)

Foreign particles suspended in the circulating blood are carried into the liver sinusoids at whatever rate blood is flowing into the sinusoids Hence the rate of supply of particles to hepatic phagocytes is faster when the inlet and outlet sphincters are open than when the outlet sphincters are closed during periods when the sinusoids are in blood storage phases, and the fastest flow rates occur when the hepatic artery is widely dilated sending blood in jetting pulses into, through and out of the sinusoids

The phagocytic von Kupffer cells do not ingest every particle which comes into the hepatic sinusoids What factors determine the selectivity of the selective phagocytic removal of particles from the blood stream? Why do the von Kupffer cells ingest some particles and miss or ignore others?

When fine droplets of undiluted Higgins India ink are injected into the blood stream, each droplet immediately receives a visible coating of a clear glassy precipitate probably protein, derived from the frog s plasma Injected particles of kaolin graphite, and mercuric sulfide also receive protein coatings The coatings often, but not always, have long feathery fibrous streamers of newly formed glassy precipitate attached The coating which entirely surrounds the ink, changes the droplet into a coated semisolid particle When a coated particle with a streamer is carried into the branching and anastomising hepatic sinusoid system the tail of the streamer may whip around a sinusoid bifurcation or may drag along the sinusoid lining the tethered particle happens the streamer sticks to the sinusoid wall The moment this is then forced against the sinusoid wall by the flowing blood, and

the streamer and clear glassy coating, with its contained ink droplet, passes instantly into the cytoplasm of whatever sinusoid lining cell or cells they touch The coated particles appear to enter the cytoplasm as quickly as two mercury droplets which are rolled against each other merge into one The von Kupffer cell may then swell a little and, depending upon the pressure on each side of it, it may bulge a little either into the sinusoid lumen, or outward indenting the adjacent hepatic parenchyma cell cord A coated particle which has no streamers may pass some distance along the sinusoid system before striking the wall hard enough to make firm contact with the lining But at what ever point it bumps or rubs against the lining, it passes instantly into the sinusoid lining von Kupffer cell cytoplasm These phagocytes have not been seen to put out pseudopods Their ingestion processes are much too fast for that, and, thus far, appear rather to have the characteristics of simple strong surface tension phenomena

Massive doses of heparin given frogs before injection of ink, kaolin, or graphite prevented the formation of coatings on particles *of these materials, and the naked particles bumped, rolled, and slid* along the inner surfaces of the sinusoid lining phagocytes without being ingested Normal frog red cells have no coatings and are not ingested

From these experiments it seems necessary to conclude that in the frogs

1 Some kinds of particles, including red cells, will not be ingested by hepatic phagocytes unless they are coated

2 The hepatic phagocytes will ingest these coatings regardless of what is in them

3 The presence of the coating determines the selectivity of the selective phagocytic removal of particles from the blood stream by hepatic phagocytes

4 The von Kupffer cells can selectively ingest coated particles from blood which is flowing rapidly, that is, the old idea that blood must be stationary or flowing slowly to permit phagocytosis is not true, at least under the conditions of these experiments

The number of particles which can be removed from blood per hour or day depends upon the rates at which particles suspended in blood are brought into hepatic sinusoids, and the coating which causes the phagocyte to select what it ingests, plus one other, the number of phagocytes which at any one time are ready to ingest particles

We can say, from these experiments, that the rates at which par ticles suspended in the blood are carried into hepatic sinusoids de termine the maximum rates at which particles can be brought to

hepatic phagocytes, and this rate together with the selective surface factors which determine that a particle is phagocytizable and the factors of chance which cause a coated particle to touch a phagocyte determine the rates at which particles are removed from the blood by those phagocytes which are at that time capable of ingesting particles

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EDITOR The above abstract is published in detail in the following reference a [illegible]

[illegible] Skrifter, Bind 4 Nr 1, 1948

A brief account of this work also appears in the following reference Knisely M H, Bloch, E H and Warner, L. A Preliminary Account of the Structure and Mechanical Functioning of Living Frog Liver Lobules, and of Microscopic Observations of the Selective Phagocytic Removal of Coated Particles from Flowing Blood by the Sinusoid lining von Kupffer cells — Conference on Liver Injury, Transactions of the Fourth Meeting, Josiah Macy, Jr Foundation, New York, October 25 26, 1945, p 21-41

DISCUSSION

Best Dr Knisely, I should like to ask a question relating to the arterio-portal anastomoses you demonstrated Has anybody ever collected a sample of portal blood and demonstrated that it contained more oxygen than the portal blood entering the liver?

Knisely I don't know I doubt if anybody has tried that

Best It would be rather interesting I was just wondering how much oxygen those anastomoses really could contribute How much increase of portal blood oxygen could you get from that anastomosis?

Knisely I am only talking about anatomy, the aspect of rate of flow must be considered The portal vein gets shut off completely and there are torrents of arterial blood, or nothing at all If one wants to go back to the environment, as far as the supply of blood through the sinusoids goes, it is pure arterial or the artery can shut off to give pure portal flow There is no difficulty in controlling the anastomoses and twigs Here the ratio of mixture is controlled rather precisely for long periods of time

Neefe For how large a portion of the liver may that appear?

Knisely I cannot tell you that One is able to visualize only two or three at a time and these are all down at the edge of the liver This method permits you to see precisely what happens A method that gives a broader perspective is that described in the paper by S Soskin, H E Essex J F Herrick and F C Mann (Am J Physiol 124, 558 (1938)) in which they used thermostromuhrs to measure the rate of flow Sometimes one finds as much as 90 percent of the blood

will be by way of the artery and sometimes 90 percent by way of the portal vein

Shorr Engel, Harrison and Long (J Exper Med 79, 48 (1944)) have shown that the rat liver is very susceptible to anoxia *in vivo*, even short periods of oxygen lack (more than 15 minutes) leading to significant reductions in oxygen consumption But in the eviscerated rat with the hepatic artery left as the only blood supply to the liver, the oxidative needs are apparently entirely satisfied, for there is no reduction in the oxygen consumptions of such livers after as long as 4 hours, suggesting that no hypoxia of significance results under these conditions

Best We must get Dr Bollman s opinion as to how well the studies with the thermostromuhr stand up in view of everything we now know

Knisely You are questioning the thermostromuhr a little bit?

Best Quite a lot, if you like

Bollman There is no question but the thermostromuhr has its limitations as well as its tribulations However, when it is properly fitted and properly checked and rechecked, accurate readings may be obtained The blood vessel being studied is not cut, but it is encircled however, loosely, and a wide range of blood flow may be determined in a given blood vessel The main point that has been demonstrated concerning the total blood flow to the liver is that it may be greatly increased or decreased under many physiologic or pathologic conditions, similar in this respect to the other organs of the body In addition both the arterial and portal blood flow may be altered to a degree independent of each other. Thus the total blood flow to the liver may be altered and the percentage supplied by the arterial and portal components differs greatly at different times, especially as influenced by the stage of digestion and exercise The overall average for the day is certainly an unknown quantity, but I would like to emphasize the extreme flexibility, both as to the total blood flow and to its component parts

Knisely There is a piece of history here which may be worth reciting Some of the first papers that came out on the percentage of blood flow in the liver were by R Burton Opitz (Quart J Exper Physiol 3, 297 (1910), 4, 93 103, 113 (1911), 5, 83 (1912)) Many of the textbooks for medical students state that from a to a third of the blood entering the liver is by way the rest is by the portal vein, these data were taken fr papers He used Ludwig's stromuhr Let job for his day and age He measured the artery in one dog or cat, and then sh

neasured the portal vein and added the two together Notice the allacy in adding together those two evidences and making a statement from the portal flow in one animal and the arterial flow from another, because it is impossible to do both at the same time The assumption was made that that happened all the time It was an assumption that physiology was a constant thing over a long period Even though the stromuhr figures may be doubted at the moment, I think we are ready to employ more delicate instruments if we can devise them We need to know the ratio of these two flows under many definable circumstances rather than with two animals

Best Indeed we do C A Crandall, in collaboration with A Lipscomb, (Am J Physiol *148*, 312 (1947)) has used the London cannula technic and his conclusions are quite at variance with those derived from the thermostromuhr work, so it is really, and I think Dr Bollman will agree, pretty much an open subject But regarding your present blood flow measurements, I suppose they are reasonably satisfactory

Bollman The blood flows at any moment may be accurately determined and I would be satisfied with the results if the thermo stromuhrs in situ were again checked by mechanically pumping the same volumes of blood through the vessels I think there is a more important feature which Dr Knisely will bring out I have heard him mention it before that the overall picture of blood flow is the summation of blood flow to the individual lobules Some sinusoids may be filled and retain blood while others shunt blood rapidly into the venous system Some lobules receive practically 100 percent portal blood and many receive different mixtures of the two The overall average of the blood flow to and from the liver is the sum of all these variables the individual components of which seem to be in continuous variation There are probably but few points within the liver lobule where the blood flow is constant and continuous with blood of uniform composition

Fremont Smith Isn t that highly pertinent to the many functions of the liver and the hundreds of chemical reactions it performs?

Bollman Five hundred

Knisely That was a guess, there are so many that all you can do is worry for the moment about some of them

Fremont Smith This variability in the same part at different time as well as in different parts of the liver is a very important facto and more important than the overall in terms of being able to unde stand what is going on

Knisely Highly controlled things! One of the most precisely co trolled things I have ever run across in the living body!

Best From another point of view, just to make an interesting controversy, what we want to know when we are studying sugar formation, is not really what happens in the individual lobules and the variation between them but the overall picture

Fremont Smith Maybe the whole liver is not participating, so maybe you want to know how much of the liver is doing this and at what rate

Best We all agree that we would like to know what each individual part is doing and also what the whole thing is doing

Markowitz May I interject a note? The portal vein derives its blood from three huge arteries, the superior mesenteric, the celiac axis and the inferior mesenteric We have to postulate for a moment that the velocity of blood flow in the arteries is the same as in one small branch, the hepatic artery Even if you did not measure directly the blood flow in the hepatic vein, you would guess that the hepatic vein would have four or five times as much blood as the hepatic artery because otherwise where is the blood going? We must not forget that If we are inclined to think the hepatic artery carries more than the portal vein, what is happening to the blood pouring into the portal vein?

Knisely It is sometimes stored for relatively long periods

Markowitz If you tied it, there is evidence of considerable blood flow which is very acute in a minute

Knisely That is true

Fremont Smith Is there a possibility of variations in the arterial supply to the portal vein which might mean that if there is a constriction of those arteries from which the portal vein blood is derived you might have quite marked variations in the amount of blood entering the portal vein? Is that tenable?

Markowitz Anyone who has done as I have done, in attempting to anastomose the splenic artery to the splenic vein recognizes how true that is In the dog you start with a splenic artery that has a diameter of a cigarette and end up with something like the end of a match The operation is practically impossible There must be a terrific variability in the caliber

Best We have also a large capillary bed in the intestine

Gyorgy And in the spleen

Best We have here the possibility of storage apart from storage in the liver

Fremont Smith May I go back to something earlier? It seems to me whether one is concerned with the overall picture or a particular

aspect, depends upon what you are interested in, and therefore there need be no controversy Obviously, as was pointed out, we need to know both In some situations we have got to know the overall, in others we have got to know the particular

Best Dr Knisely, of course, cannot give us the picture of all the lobules simultaneously If so that would be the thing

Knisely I would give up instantly on that

Best So you want to know the representative picture or the variations We also want to know what is coming out of the hepatic veins from the overall metabolic point of view

individual pieces forming a totality, and, in the liver as in the kidney, the overall picture as well as the particular aspect may become crucially important with respect to particular problems

Shorr What is suggested by Dr Knisely's description is that the liver has the appropriate vascular mechanism for purposefully altering the gaseous environment for homeostatic purposes There is nothing to say that only aerobic metabolic products and processes are of value to the organism Indeed the hepatic vasodepressor VDM (ferritin) with which my laboratory is concerned is formed by normal liver under anaerobic and inactivated under aerobic conditions It is reassuring to see that the vascular pattern in the liver is such as to permit the necessary shifts in oxygen tension which would be required by this metabolic pattern

SECTION II

THE FUNCTION OF THE HEPATIC ARTERY

Best I will ask Dr Markowitz to proceed with his presentation on the function of the hepatic artery in the dog

Markowitz When I came back to Toronto from Georgetown University in 1932 and got a position with Dr Best, I remember telling him that what I was doing was studying the hepatic artery and its function I wanted to see if one could prevent hepatic necrosis by introducing arterial blood into the portal blood and then tying the hepatic arteries I have been doing this, with interruptions owing to the war for 15 years. In 12 or 13 years I thought I had a few cases in which I tied the hepatic artery, arterialized the portal vein in one of a number of ways and the animal lived When I was on the point of repeating the crucial experiment, I always failed to confirm the finding One year I thought I could substitute for the hepatic artery by putting arterial blood into the portal vein and another year I could not

I tried to arterialize the portal vein in various ways

1) One way which was more successful than any was to do an anastomosis between the aorta and the portal vein directly side by side using the method of Eck s fistula That has a high mortality First the portal vein has to be pushed a long way down and the two stay sutures tear out More usually the mortality is due to the fact that the portal vein becomes acutely kinked and because of the high pressure of arterial blood there is enough intraportal pressure to obstruct badly the blood flow from the bowel which becomes blue It is quite a thing to see the liver rosy red and the bowel blue

2) Another method is to arterialize the blood in the vena cava by doing a side to side anastomosis of the vena cava and aorta That is easy to do That blood now being arterialized we would do a reverse Eck fistula That blood in the vena cava having been shunted into the liver we would tie the hepatic artery

3) One other way would be to join the splenic artery to the splenic vein That always failed because of thrombosis

4) Attempts to anastomose the renal artery to renal vein failed similarly

In 1918 I paid a brief visit to Mayo Clinic. There I saw Grindlay Bollman and their as Transflex tubing in their

studies on lymph This was a technic I thought I could use to join the splenic artery to the splenic vein, using the Transflex tubing, having first coated the tube with silicone Since you cannot be sure the technic is really aseptic, I thought we would give the animals some penicillin So, as we planned, we used siliconed Transflex tubing and gave the animal penicillin to make sure that the animal would not succumb to infection In every instance we were faced with the result that although the hepatic artery was effectively tied the animal stayed alive However, when we came to examine the tubing, it was always thrombosed After debating this backward and forward, we could do nothing but repeat the experiments doing a control for the penicillin We tied the hepatic artery in a number of animals, all the animals who failed to receive penicillin died All the animals who got penicillin effectively survived, except one which died of bile peritonitis

Fremont Smith With the hepatic artery tied those that were given penicillin survived?

Markowitz Yes, that is something very unusual I think you might be interested to hear something of the literature I have a fuller account of it in my book (J Markowitz, Experimental Surgery, Williams and Wilkins Co, Baltimore, 1949) S B Wolbach and T Saikr (J Med Res 21, 267 (1909)) gave the whole point a new twist when they stated that 21 out of the 23 healthy dogs they examined, had livers containing spore bearing anaerobes They used extreme methods of insuring asepsis Ellis and Dragstedt (cited from J Markowitz, A Rappaport and A T Scott, Am J Digestive Dis (1949) in press) stated that the liver normally housed spore bearing anaerobes which they thought were Welch s bacilli Boyer and McFedridge (cited from Markowitz Rappaport and Scott, loc cit) later tested these findings and decided that the cause of death from liver autolysis was the liberation of toxic material from the enzymic post mortem degradation and was not due to bacterial decomposition

There is nothing in the anatomy of the hepatic artery, as shown by Knisely s studies to indicate just why animals should die following its ligation A Nareth (Deuts Zeitschr f Chir 135, 305 (1916)) tied the hepatic artery in dogs and found they all died However, he stated that when he anastomosed the hepatic artery side to side, with the portal vein they survived ligation of the hepatic artery However I cannot repeat his operative technic and I would like to meet the man who can Nareth had three dogs out of seven that survived such arterialization of the portal vein One dog lived 12 and one lived 62 days The one that lived 62 days had a fine cirrhosis I am not satisfied with his description of the post mortem data He says nothing

about the state of the stoma except this, that when you forced a little of Teichman's fluid into the hepatic artery it appeared in the liver Whether you could dislodge a thrombus that way or not I don't know Furthermore he says nothing about the condition of the gall bladder To find that the gall bladder is gangrenous is one of the criteria that the hepatic artery has been properly tied Usually the gangrenous process becomes walled off Occasionally it does not become walled off and there is bile peritonitis If the gall bladder is not gangrenous we say a vessel was missed and when you look for it you find it every time

A few words may be said regarding the technic of hepatic artery ligation The hepatic artery gives off the gastroduodenal artery, the right gastric artery and then divides into the two hepatic branches after first giving off the cystic artery The main trunk is a pulsating loop along the portal vein When you ligate the pulsating loop proximal to the gastroduodenal and right gastric arteries, the animals invariably survive Haberer (cited from J Markowitz, Experimental Surgery, loc cit) said that in order to get necrosis of the liver after tying the hepatic artery you must tie the pulsating loop to the right of the portal vein, the gastroduodenal artery, and the right gastric. In practise, wherever you see an arterial vessel going to the liver, tie it There is one here, and there is one there, and you tie them and hope You should actually be able to see the bifurcation in the portal fissure but it is not good enough to tie only the terminal trunks because sometimes a little hepatic artery courses behind them If you tie everything in sight you invariably get hepatic necrosis

The microscopic appearance of the dog's liver following hepatic artery ligation is striking An animal operated upon 24 hours prior, and having received no penicillin, develops massive necrosis On the other hand, if the animal receives large doses of penicillin the liver appears normal The penicillin is given in the form of 200,000 units intraperitoneally as well as a large dose intramuscularly I don't think the dose is important so long as some is given

Shorr Do you think the intra abdominal route is necessary?

Markowitz I don't think so We did it for good measure

Shorr When do you first obtain demonstrable culture, in six hours?

Markowitz I cannot answer We have done them in 18 hours but we have not done them before that The organisms are anaerobes, large spore bearing bacilli On microscopic examination, the liver seems almost to be a pure culture of such bacilli

Armstrong Do you have to continue the administration of penicillin in order to keep the animals going?

Markowitz I think in some instances one injection of penicillin will do it If given only one injection of penicillin they might live five or six days We have given many injections just to make sure We have kept it up for ten days and all survived indefinitely thereafter with no further penicillin

Dr Hartroft has studied many of the sections from these animals I should like to have him comment on some of his observations

Hartroft Studies of the ligated vessels have shown fibrin in the center of the vessel * In many cases the force of the ligaturing has ruptured the internal elastic lamina Elastic tissue stains have been employed to demonstrate that and to produce evidence that the vessels are being completely obstructed

One would expect to see interesting changes in the portal canal I think surely one must after some time One sees very little in an animal sacrificed 48 hours after the hepatic artery ligation if penicillin has been employed The artery is small but, as Professor Knisely has already shown us these vessels can vary greatly in size The bile duct epithelium however, is beautifully preserved, there is no evidence of degenerative changes

There is no evident abnormality present in the central region of a liver lobule from an animal killed 48 hours after the hepatic artery was ligated when that animal was given penicillin It will be interesting to follow these animals at longer periods of time

In the necrotic liver that occurs when no penicillin is administered following ligation of the hepatic artery one sees branches of a portal vein just filled with masses of bacteria I believe these are branches of a portal vein but because the liver is so necrotic it is difficult to identify the structures with certainty

Markowitz The function of the hepatic artery so far as we can tell is to maintain the concentration of the oxygen at such a height that these organisms normally resident there don t grow We have thus far been unable to find any other function for the hepatic artery apart from the mechanical one of nourishing the gall bladder We have watched such animals for as long as 5 months

This is a new kind of homeostasis I wrote Professor Knisely to ask if he knew when the hepatic artery first appears in the animal scale Does a lobster have a hepatic artery? The liver is meant to be supplied purely by venous blood I think as you go down the lower animals you find conspicuously the portal vein drains in the liver which has developed phylogenetically as an organ capable of living at a low

* Editor Dr Hartroft presented a series of lantern slides illustrating the histological findings

or reduced oxygen tension The oxygen tension in mixed venous blood is I think 42 mm of mercury The mixed arterial blood is 72 mm of mercury The latter oxygen tension corresponds to that which obtains at a height above the earth of about 6 5 kilometers and a tension of 42 mm of mercury corresponds to that at about 10 kilometers Translate that and think what would happen in the pulmonary alveoli if we were breathing that sort of air It would be the difference between Pike s Peak and Mt Everest We can live at Pike s Peak but I don t think we can live well at Mt Everest

Dragstedt and his school have for years maintained that the liver harbors large numbers of anaerobic bacteria When necrotic liver tissue is left about these proliferate Thus in a sense the animal dies of gas gangrene in the liver

We won t have it that when you tie the hepatic artery little areas of gangrene develop in the liver which permit the spores to germinate It is an obvious enough thing to say the spores in such areas germinate and once they get started nothing can stop them Hartroft has looked carefully for such areas of gangrene and there are no such areas The liver looks remarkably normal three months after this operation is not markedly reduced in size and is unscarred

Madden Penicillin is just given for the first ten days but the animal lives for three months?

Markowitz Lives indefinitely Whether it needs to be given for ten days or three days I don t know

Madden Aren t they reinfected with these organisms?

Markowitz That is a thing which has puzzled me Maybe they become immune

Madden You don t get reinfected to tetanus and typhoid

Shorr What about the collateral circulation?

Markowitz That is being studied by Hartroft He can enlarge on that

Shorr We have seen that

Markowitz Maybe they get collateral circulation but the fact is they die without the penicillin and live after they have it

Shorr After that period they develop a collateral circulation We have noticed that after some months

Markowitz Where the musculo phrenics?

Shorr Through the diaphragm

Markowitz As soon as an animal dies or as soon as we kill it Hartroft does a careful autopsy He makes sure the ligatures are

tight Whether the animals develop a collateral circulation in the ultimate future is another problem It is very likely they do

Patek Have these organisms been shown to be penicillin sensitive in culture?

Markowitz The Welch bacillus is known to be sensitive The soldier and gas gangrene who is given penicillin has rapid healing of his infection Whether this is the Welch bacillus, or is a variant, as the bacillus of hog cholera is a variant of the human, I don't know I think the organism is either a Welch bacillus or close to it Some people say they actually are Welch bacilli

Best. Really the problem has been do the dogs die without penicillin if you tie the hepatic artery and do they live with it? There are

At a recent lecture in Toronto one of the surgeons stated that he had once, like many other surgeons, inadvertently tied the hepatic artery and the patient had died The physiologists looked smug They know how to save an animal when they tie the hepatic artery If this situation should arise again I think the surgeon would want to give the patient all the penicillin he could I don't know whether the patients would live but I know the dogs do

Watson I want to know to what extent this could be transferred to human beings

Markowitz Quite directly

Watson I had the impression that the human liver does not normally contain resident spore forming bacilli, anaerobes

Markowitz I merely quote Dragstedt I directly have no interest or authority in that problem

Watson Are you acquainted with the studies of M H Manson (Thesis A Consideration of Some Factors Responsible for Gas Bacillus Infections University of Minnesota, 1934)? He worked on this problem quite extensively and my recollection of the study is to the effect that he regularly got the anaerobes in dog livers but hardly ever could get them in human livers

Markowitz This is a non sequitur Why do human beings die when you tie the hepatic artery?

Best That could be from quite a different cause

Watson I think that deserves a lot of discussion The problem of hepatic artery obstruction in human beings is one of infarction of the liver

Markowitz Total infarction?

Watson No For example we saw a case a few months ago of an individual who had a cancer of the pancreas with involvement of the hepatic artery It appeared not to be completely obstructed although the lumen was not more than a pin point That patient had multiple infarcts in the liver Also in periarteritis nodosa of the hepatic artery one sees complete obstruction with thrombus secondary to the disease together with multiple infarcts in the liver

Markowitz Where is the obstruction in the portal lumen?

Watson It varies in location but it may be up in the liver in the branches of the hepatic artery with multiple thrombi but there may be multiple infarction in the liver here as well As I understand it the process in the dogs is entirely diffuse and they don t have any infarcts

Markowitz Years ago I tried to tie one of the two main branches of hepatic artery You would think no problem would be simpler I did two dogs and both lived I gave up If you want an explanation Professor Knisely supplied it There is enough arterio venous anastomosis You have retrograde flow

Best We must bring in the rats If you tie their hepatic artery they don t die Dr Arthur Colwell Jr in my laboratory has confirmed this fact but one of his rats did die with a gangrenous liver

Fremont Smith I would like to ask if you have infarcts as in the case you have spoken of would that not be the ideal situation for at least the local growth of the anaerobic organisms and that therefore the absence of such growth would be some evidence against their presence in the human where infarcts had been seen? Is that a reasonable line of approach?

Best Just to set the discussion a bit straight perhaps the situation is if you tie the hepatic artery in the dog you get a liver that is full of these organisms It goes on to almost total necrosis does it not? The situation is absolutely different in the rat where if you tie the hepatic you don t usually have to give penicillin at all When the hepatic artery has been inadvertently tied in the human beings the picture is obviously quite different than that in the dog or in the rat Therefore none of us know the application of these results to the human being

Markowitz There is a case by R R Graham and D Cannell (Brit J Surg *20* 566 (1933)) in which they accidently tied the hepatic artery I think the patient died of total necrosis of the liver

Armstrong With reference to your remark about the rat this animal is notably resistant to many sorts of infections The fact that

the rat s liver does not show any necrosis from the anaerobic organisms might be the result of this mysterious resistance which this animal certainly possesses with regard to pyogenic infections

Markowitz I suggest that that is the explanation

Short The presence of anaerobic organisms normally in the dog liver calls to mind the experiments of Aub and his colleagues on the possible role of bacterial toxins in traumatic shock They partially detached skeletal muscle of the hind limb of the dog, encased it in a sheath and constricted the limb proximally They collected the exudate of the muscle preparation after about 9 hours and injected it into normal dogs It produced a toxic type of shock The exudate was then shown by them, in collaboration with Dubos, to consist of a rich culture of Clostridium welchii It is uncertain whether the organisms were harbored in the skin or muscle From work in our own laboratory we suspect the skin, since anaerobic preparations of dog skeletal muscle in vitro removed with strict asepsis, failed to show these organisms and indeed very few bacteria after 5 hours in nitrogen

Best Dr Bollman have you something to contribute here?

Bollman I have two things I would like to mention First I want to thank Dr Markowitz for doing this nice piece of work I have been worried about the importance of the hepatic artery for years and have considered the use of Welch antitoxin to accomplish the same thing that Dr Markowitz has done with penicillin I am not sure that Welch antitoxin would be as effective but it is not necessary now to attempt the study

The other thing I would like to comment on is the subject which has already been mentioned the collateral circulation to the liver After reading Dr Markowitz s paper in the Proceedings of the Society for Experimental Biology and Medicine we ligated the hepatic artery and gave penicillin and the dogs lived just as he says they do Not all of the arterial blood to the liver enters by way of the hepatic artery There are two additional places where small arteries enter the liver one from the circle around the attachment of the vena cava to the diaphragm and the other from the lower side of the liver near the entrance of the portal vein This supply in the dog is not sufficient to maintain the liver free of lethal infection (Slide demonstrated) This slide was made four days after ligation of the hepatic artery of a dog which had received penicillin and appeared well at the time The animal was anesthetized and I tied the aorta in the chest and just above the renal arteries before injecting the barium acacia mass This x ray plate shows the distribution of the barium in

Obviously it does not go the whole route Something happens when you tie the hepatic artery completely in the dog It strikes me as peculiar that when you increase the rate of venous blood flow, the blood still has a lot of oxygen, but you do not keep these animals alive You can get a tremendous flow We have been talking about the dynamics and the difference between arterial and venous tension If you increase the rate of flow you can make a lot of oxygen available There is something very fundamental in this survival after ligation

Fremont Smith I wonder if the shock of the operation puts the animal for a short time immediately after the operation in a state where with the hepatic artery tied off, the organism can start to grow in the liver Then there is the fact that a short course of penicillin, probably too short to allow full collateral circulation to develop still allows survival and shows that there we are dealing with a temporary condition Are the blood pressures of these animals low immediately after you tie the hepatic artery?

Markowitz You mean where we just tie without the Eck's fistula? They are normal animals

Fremont Smith There is not very much shock?

Markowitz There is no shock

Fremont Smith Anesthesia does things Anesthesia is nearly always followed by something

Markowitz We did two kinds years ago I quoted my teacher, Dr Mann, who says that ether is the best anesthetic With the greatest of respect we switched over to nembutal as it is easier There seems to be no greater shock than with ether Naturally they sleep longer with the nembutal

Fremont Smith Both are quite bad for the liver, both the barbiturates and ether They do knock out a good many liver functions

Best I might say we were anxious to check these experiments and at Dr Markowitz suggestion and in quite a different laboratory the same experiments were carried out, ligating the hepatic artery We picked a young surgeon from the department, a very skilled man and he failed several times to get his animals to die after ligating the hepatic artery It turned out that he did not know how to tie the hepatic artery in dogs, and again and again left a little branch There is something more than just talk when Markowitz says he ties every thing he sees You more or less have to do this Even a man who operated a great deal on human livers and biliary tracts is not an expert in the case of the dog until he has had a lot of experience

Bollman Dr Best has touched on one thing which I think would be well to bear in mind We have mentioned the oxygen supply by the hepatic artery, but we have little evidence that the loss of oxygen is the major factor Fremont Smith mentioned that other things may be in there The cons

sinusoids as we saw

arterial supply was

probably continue to fill the sinusoids and distend them to occupy the space formerly held by arterial blood From the appearance of the liver I would suspect that the emptying of many of the sinusoids was delayed and that considerable stasis developed The oxygen content of the portal blood is low and is undoubtedly much lower when stasis has occurred in the sinusoids The same could be said of the nutritional elements of the blood and the removal of metabolites may be impaired I do not like to blame all the changes on the lack of oxygen until we have something more definite about oxygen and the other factors involved Many articles in the literature use the term *reduction of oxygen supply interchangeably with arterial ligation* and in some instances I am sure there has not even been a reduction of the amount of blood flowing to the organ involved

Watson Could you control that by running portal blood through the oxygenator for the first 24 hours? I don t know how difficult it would be technically

Bollman It would be difficult but it could be done

Fremont Smith Is there enough glucose? What is the glucose content?

Bollman Less than in the arterial blood

Fremont Smith Oxygen has to have something to utilize and I wonder whether there is enough for it to utilize

Best The glucose content is not lowered This oxygen deficit is I believe just a good guess at this stage

Markowitz I am trying to reason in the most elementary fashion I can If you double the blood flow by reverse Eck fistula dogs die when you tie the hepatic artery even though the liver is getting more blood than ever

Bollman The fact that the animals survive long after the penicillin has disappeared indicates that something has happened during the period after arterial ligation and the elimination of the penicillin I doubt that sufficient collateral arterial circulation has developed to supply oxygen equivalent to that formerly supplied by the hepatic artery

Armstrong Coming back to oxygen, I don t know what the oxygen tension of portal blood is Will it differ greatly from mixed venous blood or not? If it does not, there is still a lot of oxygen in that blood and much higher amount than the bacteriologists can tolerate when growing Welch bacilli

Best I don t know what the oxygen tension of the portal blood of the dog is under these conditions and even less what the oxygen tension of the cells in which the organisms are growing They are growing in the cells I presume

Armstrong The cells should have oxygen tensions somewhat related

Watson Probably much lower

Best We don t know how much the normal hepatic blood flow raises the oxygen tension of the cells

Madden You can put a catheter in the hepatic vein and study all the variations

Best If we did all the things Markowitz wants us to do we would not do anything else in the department

Stetten If the degree of anaerobiosis is sufficient in the cell to permit the growth of Welch bacilli it may be that some of the oxida tive functions are not operating like the one which converts beta carotene to vitamin A which is definitely an aerobic liver function

Shorr The blood uric acid and ammonia levels furnish a good index in the dog as to whether the metabolic processes in the liver are oxidative or anaerobic The blood constituents are kept at normal levels as the result of oxidative processes within that organ When oxygen tensions are sufficiently reduced to initiate an anaerobic type of metabolism the blood uric acid and ammonia values rise The use of these criteria in your experiments would help determine the extent to which anaerobiosis occurs and its duration

Best This growth of bacteria might be really secondary to some other primary change caused by the procedure

Markowitz Which is non fatal

Best It is not fatal The study of the liver after complete ligation of the hepatic artery in dogs receiving penicillin is a fascinating thing For the first time you have got dogs without an obvious arterial blood supply to the liver

Bollman I am not sure that you do have a liver deprived of arterial circulation I do know that there is a little arterial circulation remain ing I wish you would continue these studies and find out whether this collateral arterial circulation develops with time or always remains small

Best I don t know much about this but it seems to me you have a little knob of arterial circulation near the diaphragm from another source than the hepatic artery How much this develops after hepatic artery ligation I don t know

Hartroft May I ask a question? Coming back to Dr Knisely have you in any study seen direct connections between the hepatic system veins and the portal system veins?

Knisely I have never seen any a sinusoid can dilate very widely It may be as much as 200 micra Whether that is pathological or just physiological I don t know

Bollman With injection technics some direct communications be tween the branches of the portal vein and the hepatic veins have been demonstrated I have seen them very clearly I suspect that you have similar connections in your photographs where you have the many branches of the portal vein going in all directions an occasional branch seems to directly join the hepatic vein

Hartroft I believe I have seen them in cirrhotic liver

Bollman They are very marked in cirrhotic livers You may have quite a large anastomosis direct from the portal vein to the hepatic vein

Knisely M Prinzmetal E M Ornitz Jr B Simkin and H C Bergman (Am J Physiol *152* 48 (1948)) have been inject ing glass beads in animals and catching them on the other side I don t know whether they found they were large enough to pass them

Watson They did

Hoffbauer Spheres from 60 to 180 micra were recovered from the lungs of rabbits after such spheres were injected into the portal vein

Fremont Smith Another suggestion for your consideration is to put a Goldblatt clamp on the hepatic artery and turn it down gradually and see how rapidly you could bring it down without getting the infection

Shorr I can tell you what happens under those conditions as we have been studying the effects of partial constriction of the hepatic artery by Goldblatt clamps in our laboratory during the past 3 years After two to four months such an extensive collateral circulation develops to the liver from the diaphragmatic vessels that it is neces sary to go in and tighten the clamp and even completely constrict the hepatic artery to maintain the animal in the condition initially induced by the constriction We cannot say how soon an extensive collateral circulation develops after partial constriction of the hepatic artery but we suspect it occurs quite early

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Gyorgy May I make one remark? You discussed the phylogenesis of the hepatic artery in the role of the arterial blood for the liver As a pediatrician I may call your attention to the fact that the fetal liver has quite a lot of arterial blood Therefore it must play a role in addition to anything else

Markowitz I have considered that I thought, now the fetal liver has twice as much arterial blood, maybe we can put in twice as much in the adult It cannot be done On that point of view I will stake my right arm The junction flaps around, and thromboses So while you can accomplish such vascular anastomoses in the groin or neck, or in an Eck s fistula, because the parts are stationary, you cannot do so in the portal system

Gyorgy Certainly the very large liver is connected with this fetal circulation

Best We have been tremendously interested recently in rats put on diets low in choline after the animals have been starved We have seen hemorrhagic kidneys in old rats after starving and refeeding them We have seen it and Salomon has seen this too I have been wondering this morning if you did a partial hepatectomy and when that animal recovered and when the liver was presumably regenerating very rapidly on a good diet, if you tied the hepatic artery and gave the animal penicillin would you save him? There might be a demand for something which would cause death under those conditions in spite of penicillin

Markowitz That would be a good experiment One of the first things which has to be done is to see whether such a liver, with a hepatic artery tied shows regeneration or does not show regeneration after removal of portions of the liver I suspect it will regenerate

Let us call these dogs without the hepatic artery but with the portal vein as having a portal vein liver *Let us call dogs without* a portal vein flow to the liver as having an 'hepatic artery liver Finally if the blood flow to the liver is intact, let us describe it by saying the animal has a portal vein and hepatic artery liver

Fremont Smith You mean that those dogs with only the portal vein intact would show regeneration?

Markowitz The dogs whose liver is nourished by the portal vein will, I suspect show hepatic regeneration after removing chunks of the liver

Fremont Smith I thought you said already that has not happened

Markowitz In the Eck s fistula dog the liver does not regenerate In the terminology I propose, let us call that animal a d with a hepatic artery liver

Goldblatt In the light of what you told us today, would you not say that no one else before you has ever produced a portal vein liver? If you insist that a dog dies if the hepatic artery is occluded then no one before has produced a portal vein liver Is that incorrect?

Markowitz That is correct

SECTION III

MASSIVE LIVER NECROSIS

Gyorgy From a pathological point of view necrosis and cirrhosis are the characteristic and specific manifestations of injury to the hepatic parenchyma Fat infiltration *per se* is not specific enough, and often too transient, without concomitant manifestations of tissue reaction, to be considered in itself as a truly pathological condition of the liver

Today we are discussing the etiology of acute necrosis, which can be massive or zonal The production of experimental hepatic necrosis (massive or zonal) dates back many years and decades Such acute injury of the liver was looked upon as the consequence of a *toxic* influence on liver tissue by numerous noxious agents Such factors, most diverse in character, included various chemicals, both organic and inorganic, such as carbon tetrachloride, chloroform, phosphorus, etc, certain drugs, foreign proteins, bacterial products, immune serums and infections From these experimental studies, which in general, tally well with clinical observations, it was concluded that in every instance in which the prolonged or repeated "action of an agent has resulted in some degree of cirrhosis, the acute effects have been degeneration and necrosis of hepatic cells (V H Moon, Arch Path, *18*, 381, (1934)) From this point of view, necrosis and cirrhosis are the acute and chronic forms of hepatic injury, often in response to the same etiologic agent The response of the hepatic tissue to insults of various kinds manifests itself in necrosis when the insult is overwhelming, or when the resistance of the hepatic parenchyma is reduced below the norm whereas continuous or repeated exposure to the same agent given in doses which are in general below the necrogenic level results in cirrhosis When cirrhosis follows interrupted, repeated exposures of hepatic tissue to a noxious agent, it may be inferred that the injurious effect is achieved by proper timing of the insults which precluded the complete repair of the hepatic parenchyma between consecutive exposures without ever producing histologically demonstrable necrotic changes, and reduced the normally high regenerative power of hepatic parenchyma This cirrhosis cannot be distinguished in many cases from ordinary Laennec s cirrhosis These well established facts should be kept in mind because it makes it very difficult to distinguish completely even in dietary necrosis or

dietary cirrhosis two phases of specific liver injury That is not on the program of today

The question is Is there a dietary hepatic necrosis? Can it be produced? Can it be produced at will?

In 1939, Dr Goldblatt and I had published a paper on the occurrence of acute necrosis in rats kept on a semi synthetic diet not complete in various members of the vitamin B complex In this group of observations acute necrosis was not produced at will but was found in a relatively large percentage of the experimental rats (in 48 out of 300) It was never encountered in rats receiving the same basal diet supplemented with yeast extract

With the use of diet rations in which the level of protein (casein) was kept at 10 percent or below hepatic injury became a regular occurrence produce [1] experimental period up to 150 about 120 to 150 gm (or more was reached and published almost simultaneously by four groups of investigators (cf P Gyorgy and H Goldblatt J Exp Med 89 245 (1949)) In these experiments liver injury manifested itself either in the form of acute diffuse necrosis or of slowly developing diffuse fibrosis reminiscent even in the gross of Laennec s hepatic cirrhosis In some rare instances zonal or massive acute necrosis and cirrhosis were encountered in the same animal

It is not surprising that necrosis (zonal or massive) and cirrhosis produced by dietary means were first considered in the same relation to each other as the corresponding pathological changes in the liver occurring under the influence of noxious agents such as chloroform carbon tetrachloride etc It has been assumed that necrosis is only the acute manifestation of a quantitatively more severe insult, than that causing cirrhosis which follows chronic and moderate hepatic injury A closer analysis of the etiologic dietary factors found in acute necrosis and in cirrhosis furnished results which seemed to be at least to some extent at variance with the unitarian theory of their etiology It was observed that supplements of choline or liver extract to the experimental diet would prevent cirrhosis but were without influence on or would even promote acute diffuse necrosis Conversely cystine given as supplement to the experimental diet exerted a remarkable protective effect on the development of necrosis but seemed to enhance the production of hepatic cirrhosis On the other hand methionine or protein rich in methionine prevented both acute diffuse necrosis and cirrhosis Protein low in methionine and at the same time rich in cystine such as peanut protein will prevent necrosis

but will enhance, even when added to the diet in high relative proportion, the development of cirrhosis The rather complicated interrelations and effects of protein, methionine, cystine and choline as well as of other dietary factors (fats and vitamin E) to be discussed later, are summarized in Table I

TABLE I

Effect on	*Protein* (Methionine Containing)	*Methionine*	*Cystine*	*Choline*	*Vit E*	*Fat*
Cirrhosis	Beneficial	Beneficial	Injurious	Beneficial	0	Injurious
Massive Necrosis	Beneficial	Beneficial	Beneficial	0 or Injurious	Beneficial	0 or Injurious

F S Daft, W H Sebrell and R D Lillie (Proc Soc Exp Biol and Med, *50*, 1, (1942)) as well as Himsworth and Glynn (cf Himsworth, H P, The Liver and Its Diseases, Blackwell and Co, Ltd, Oxford, (1947)) have sharply distinguished massive hepatic necrosis from diffuse fibrosis (cirrhosis) They considered massive hepatic necrosis as a sign of cystine (methionine) deficiency, and cirrhosis more as a result of a deficiency of choline and its precursors, including methionine

With the addition of choline to a diet low in casein, cirrhosis may be completely prevented but the incidence of necrosis, although accelerated and intensified was increased only slightly The irregular occurrence of massive necrosis in rats fed a diet low in casein was claimed by Himsworth (loc cit) to be due to the quality of the casein which even when given in small amount supplied methionine (and cystine) not too far below the prophylactically effective level In this connection it should be pointed out that the hemorrhagic foci ob served by T E Weichselbaum (Quart J Exp Physiol *25*, 363, (1935)) in the livers of rats fed a cystine deficient diet may have represented necrotic foci, but were not described in detail or recog nized as necrosis

By substituting yeast for casein the intake of sulfur containing amino acids may be further lowered Thus, it seemed to fulfill ex pectations, when reports appeared (H P Himsworth, loc cit, A Hock and H Fink, Ztschr physiol chem, *279*, 187, (1943)), that in rats fed rations with yeast as the sole source of protein massive necrosis became a regular occurrence According to these observations the development of hepatic necrosis was again completely suppressed by prophylactic supplements of cystine or methionine

These experiments bring us up in chronological order to 1943/44 Our original basal diet contained casein (8 10%), and, as fat, lard (20 23%) with cod liver oil (2%) mixed with the ration In rats fed this low casein high lard diet, acute necrosis developed in about 40 50% of the experimental animals Substitution of percomorph oil, 3 drops once a week, for cod liver oil mixed with the diet or of butter fat for lard reduced markedly the incidence of acute necrosis in rats kept on a low casein diet Thus, unsaturated fatty acids, as present in cod liver oil and the lard, seem to enhance the development of acute hepatic necrosis (See Table 1) In 1942, due to wartime restrictions we had to substitute hydrogenated cottonseed oil (Crisco) for lard From that moment on necrosis became conspicuous through its absence In studying the possible cause of the sudden disappearance of necrosis, we tried various other fats, and we always ran across one correlation, namely the vitamin E content of the fat It appeared, and evidence for this conclusion was presented at our Conference in 1947 (Liver Injury Transactions of the Sixth Conference, Josiah Macy, Jr Foundation New York, (1947)) that vitamin E will prevent the development of dietary acute necrosis in rats receiving a diet low in sulfur containing amino acids

Similar observations were presented during the war, unbeknown to me by Dr Schwarz from the laboratory of Professor Richard Kuhn, Heidelberg, Germany (K Schwarz, Ztschr physiol chem, *281*, 101, & 109 (1944)) I am delighted and I am sure I can speak in the name of our whole group, that Dr Schwarz is with us today and will participate in the discussion of the topic in question

Dr Schwarz used in his experiments a special alkaline treated casein By feeding young weanling rats a semi synthetic diet similar to ours with the alkaline treated casein as the sole source of protein, massive necrosis of the liver developed in a large percentage of the experimental animals In contrast to our observations cystine and methionine were found by Schwarz ineffective in the prevention of this type of hepatic necrosis while on supplements of vitamine E no necrosis of the liver developed We have tried to repeat the experiments of Dr Schwarz but were unsuccessful (P Gyorgy and H J Goldblatt Exper Med 89 245 (1949)) This negative result was due probably to some differences in technic and perhaps even more to the different casein samples used

As stated before in our observations the occurrence of acute necrosis never reached higher figures than 40 50% when rats were kept on a diet low in casein and free from tocopherol This contrasted with the findings of Hock and Fink as well as Himsworth

(loc cit), who reported a practically 100% *incidence of massive* necrosis in rats kept on a diet in which yeast furnished the only source of protein In order to solve this apparent discrepancy we carried out a large number of experiments using yeast rations in several com binations, such as high and low in yeast, in various fats (lard, Crisco), with or without cod liver oil, with or without tocopherol and/or cystine The yeast was a Type 50B dried Brewer s yeast of Standard Brands Inc Again, the highest figure for incidence of *massive necrosis*, on a low yeast high lard cod liver oil ration (without cystine or tocopherol) was only about 40% A further group of 35 rats was put on a ration modeled after the experimental ration of Professor Himsworth (personal communication) American (Type 50B) yeast 18, corn starch 79 salt mixture, 3 Arachis oil 0 5 ml and cod liver oil, 2 drops, were added to 8 gm of the dry mixture just before feed ing Further all animals received daily the usual vitamin B supplements on this diet All our experimental rats remained healthy, and at the end of the experimental period (200 days) showed at autopsy no signs of acute or healed hepatic necrosis Subgroups of these rats *were exposed to various forms of stress (injections of adrenocortico* trophic hormone, cold, exercise), and still no hepatic necrosis de veloped

In the light of these completely negative results it appeared to us as the only logical conclusion, that the discrepancy between our ob servations and the positive findings of Himsworth might have been due to the fact — other conditions having been more or less equal —, that different yeast brands were used in the respective rations Through the kindness of Professor H P Himsworth we obtained the same type of British Baker s yeast (United Yeast Co, Ltd, London) which was used in Professor Himsworth s laboratory Fermentation tests showed no activity for the American yeast whereas the British yeast exhibited slight but definite activity The British yeast was given in form of the same ration as stated above for the American yeast to *three groups* of rats.

Out of 18 rats fed the basal ration with the British yeast as the sole source of protein 15 animals died between the 100th and 145th and 2 more animals died between the 150th and 200th experimental day from acute hemorrhagic necrosis In the whole group only *one* rat survived the experimental period of 200 days and showed at autopsy normal liver The massive character of necrosis extending *over many* lobules and even lobes distinguished it from the usual more zonal form of necrosis (central and midzonal) seen in rats fed rations low in casein In all macroscopic and microscopic details the

findings conform to those given by Himsworth for his animals ke under identical conditions It was the first time that in our laborato massive necrosis was observed as a truly regular occurrence In tw subgroups of animals consisting of 11 and 13 rats respectively, anc fed the same basal diet (with the British yeast) supplements of tocopherol (3 mg daily) or cystine (50 mg daily) prevented the development of hepatic necrosis throughout The experimental period was extended to 200 days

All these experiments with the two yeast rations were carried out concurrently with rats of the same strain (Sprague Dawley) age, and identical dietary history

Acute necrosis as it appeared irregularly in rats fed a diet low in casein, and with impressive regularity in rats fed a ration containing a particular brand of British yeast as sole source of protein may be prevented either by supplements of sulfur-containing amino acids or of tocopherol These observations are difficult to reconcile with the previously held view which identified massive hepatic necrosis with deficiency of cystine (methionine) Furthermore under special conditions such as in the experiments of Dr Schwarz as well as in those recently reported by E L Hove D H Copeland and W D Salmon (Fed Proc 8 386 (1949)) cystine and methionine were ineffective in the prevention of massive hepatic necrosis which however still responded to the prophylactic administration of tocopherol In the experiments of Schwarz an alkali treated casein and in those of Hove an oxidized (methionine free) casein was used This may explain the difference in response to cystine or methionine between these experiments and those in which untreated regular casein served as source of protein

The important role tocopherol plays in the etiology of massive hepatic necrosis and the fact that the body holds tenaciously to its tocopherol stores may explain to a large extent the difficulties several authors encountered in their effort to produce hepatic necrosis in rats Thus the pre experimental diet and its duration should exert a decisive influence on the development of massive hepatic necrosis (H P Himsworth and O Lindan Nature 163 30 (1949)) The preparatory period may be reduced to 14 21 days (mean 23 2± S D 8 36 days) by feeding the usual yeast diet to young weanling rats (23 days old) whose mothers were put 10 days after the litters were born on a vitamin E free diet When the female rats with young litters were kept throughout the whole period of lactation on the usual stock diet containing vitamin E the development of massive acute necrosis in the young rats receiving the yeast diet is prolonged

to 60 days or more (personal communication of Professor Himsworth)

The peculiar interrelation between cystine (methionine) and tocopherol in the prevention of acute massive and zonal hepatic necrosis requires further elucidation The interchangeability of sulfur containing amino acids (cystine, methionine) and vitamin E as leading etiological factors makes it difficult to accept pure deficiency as the basis of acute diffuse hepatic necrosis Furthermore, it has been amply proven that cystine when given in excessive doses (M X Sullivan, W C Hess and W H Sebrell Pub Health Rep U S P H S 47, 75 (1932), D P Earle, Jr and J Victor, Exp Med, 73, 161 (1941)) and often even not too greatly (A C Curtis and L H Newburgh, Arch Int Med, 39, 828 (1927)) in excess of the physiological norm will not prevent but in fact will promote the production of massive hepatic necrosis Methionine and cystine are known as detoxifying agents in counteracting the noxious effect of hepatotoxic substances and related poisons Recently similar observations were reported with regard to the effect of vitamin E in the particular case of carbon tetrachloride poisoning (E D Hove, Arch Biochem, 17, 467, (1948)) In rats kept on the usual low casein high fat diet supplemented with the carcinogenic dye N_1 N_1 dimethylaminoazobenzene (butter yellow) acute diffuse necrosis reached a much higher incidence than in control groups fed the same diet without butter yellow Nevertheless in both groups supplements of cystine or tocopherol prevented the development of necrosis equally well (P Gyorgy and H Goldblatt loc cit) This may indicate that cystine and tocopherol neutralized first the effect of the exogenous poison butter yellow and simultaneously also that of some unrecognized endogenous metabolic poison Acute diffuse necrosis caused by large doses of cystine should also be considered as a toxic manifestation, perhaps initiated by the disturbed normal ratio of cystine and choline (P Gyorgy Am J Clin Path 14 67 (1944)) In this connection one should recall the enhancing effect of choline (also liver extract), on the production of hepatic necrosis in rats fed a ration low in cystine and methionine

The phenomenon of hepatic necrosis provides an impressive illustration of the difficulty encountered in distinguishing between mechanisms based primarily on deficiency or intoxication These mechanisms may be thoroughly interwoven and do not permit a distinct separation of the causative factors

This brings us back to the question as to whether the interchangeability of cystine (methionine) and tocopherol may be primarily due more to an underlying detoxifying mechanism than to the restitution

of a deficiency condition If this were the case, then the regular occurrence of massive hemorrhagic hepatic necrosis seen in rats on the special British yeast ration might be traced either to presence of a toxic factor or to the low content of a special detoxifying constituent in this yeast

The chemical analysis of the American and British brands of yeast is, of course, essential as a preliminary possible answer to the question What is the difference between these two brands of yeast? The analytical data for N and total S were

	N%	S%
American yeast (Fleischmann s Type 50B)	7 83	0 86
British yeast (United Yeast Co Ltd London)	6 76	0 65

The greater analytical value for total S in the American yeast may indicate a higher content of cystine (or methionine) than that present in the British yeast The further possibility that the British yeast being an active yeast, might be less well digested and utilized (E L. Price M M Marquette and H T Parsons J Nutr, 34, 311 (1947)) than the inactive American yeast received no direct support in the experiments of Himsworth and Lindan, who found no difference in the necrogenic effect of the British yeast when given alive or heat killed (personal communication of Professor Himsworth) The average food intake in the group of rats fed the diet with the American or British yeast was nearly equal (in the average 6 5 gm daily in the first 14 weeks for the group with the American yeast and 6 3 gm daily in the first 14 weeks for the group with the British yeast) On the other hand the gain in weight during the total experimental period was distinctly higher in the group of animals receiving the American yeast than in the group of animals kept on the diet with the British yeast as source of protein Even when all these various factors are considered the difference in sulfur intake about 25 percent does not appear to be distinct enough to give a satisfactory explanation for the *complete* absence of hepatic necrosis in one group and for its *regular* occurrence in the other Further A Hock and H Fink (Z Naturforsch 2b 187 (1947)) reported recently on a similar greater growth promoting activity of Brewer s yeast compared with that of Baker s yeast Nevertheless the incidence of massive hepatic necrosis was apparently equally high in both groups of their rats Obviously direct analytical data are necessary in order to express the S-values for either of the yeast samples as cystine or methionine Such analytical work is at present in progress However it is worth pointing out that in previous experiments (P Gyorgy and H Goldblatt loc cit) rats

kept on a ration containing lard and American yeast as sole source of protein and in which the yeast intake was less than half as compared to the rats receiving the ration with the British yeast, the incidence of acute necrosis, in a rather milder form, barely reached 40 percent It should also be added, that in rats of both groups receiving British or American yeast, severe ulcero papillomas (to be described later in a separate communication) of the fore stomach were noticed which were absent in the groups receiving a supplement of cystine This indicates that even the American yeast could not have contained the necessary physiological minimum of cystine (methionine?)

The other question which has been raised at the meeting of the Army Liver Commission by Dr Watson a few months ago, concerns the possible difference in the vitamin E content of the American and British yeast Generally, it is assumed that yeast is free from vitamin E All the yeast brands examined chemically in the laboratories of Distillation Products, Inc (Rochester, N Y) have been found according to personal communication of Dr P L Harris, to be free from tocopherol At least chemical isolation procedures did not give any indication for the presence of tocopherol

The possibility, although perhaps remote, that yeast might contain some tocopherol like substance which cannot be extracted in the usual way has been investigated by a new technic We have recently shown (C S Rose and P Gyorgy, Fed Proc *8*, 244, (1949)), that if a rat (we used rats of the Sprague Dawley strain) fed on a usual mixed grain diet is put on a vitamin E free diet, consisting of casein, salt, sugar, vitamins (with or without fat), beginning deficiency may be demonstrated after 3 7 days Red blood cells of such rats, washed and suspended in buffered saline solution will be hemolysed by minute amounts of dialuric acid less readily by ascorbic acid or cysteine This hemolysis may be prevented by tocopherol added in vitro to the assay mixture Further, hemolysis will never occur — and I underline the word never — when red cells were taken from rats receiving tocopherol in their diet We have put rats on the usual necrogenic semi synthetic rations containing American and British yeast respectively and after 3 days we examined the red cells of the rats to see whether or not they would be hemolyzed by dialuric acid Hemolysis occurred with equal intensity regardless whether the blood was taken from rats receiving American or British yeast Thus, both brands of yeast must be essentially free from tocopherol

The fact that yeast appears to be particularly conducive to the development of massive hepatic necrosis may be due not only to a deficiency of protective (detoxifying) factors, such as cystine (methio

nine) and vitamin E, but perhaps also to the presence of some unidentified toxic factors in the yeast, or — more probably — to the directly or indirectly supported production of some toxic metabolites. Such toxic substances may originate in the intermediary metabolism, or under the influence of the intestinal flora, in the intestinal tract, in particular in the large intestine. In any case, British yeast should promote the production of such toxic factors.

As first pointed out by Himsworth (loc cit) the necrotic changes in the liver seem to prefer the left liver. Himsworth saw in this difference a circumstantial evidence for the so-called trophopathic etiology of the condition. Blood from the small intestine supplies mainly the right liver. Protective food constituents cystine, methionine — and as we know now — also vitamin E are absorbed from the small intestine and directed to the right liver. Blood from the large intestine must be obviously low in or free from such protective food factors and in consequence, the deficiency condition — necrosis — should be more pronounced in the left liver. On the other hand if toxic products originate in the large intestine, the left liver should show more signs of injury than the right liver.

All these are, of course, purely hypothetical considerations. However, as clinician I am impressed by the fact that a pure deficiency necrosis of the liver is unknown in clinical medicine. The interplay of toxic factors tallies certainly better with clinical observations as far as hepatic necrosis is concerned. Necrosis associated with viral hepatitis is the most common form of this acute hepatic injury in the clinic. Various chemical poisons, such as carbon tetrachloride, phosphorus, the unidentified cryptogenic poisons such as in eclampsia are other common etiological factors in the development of hepatic necrosis.

As detoxifying agents, vitamin E and cystine will probably act through the intermediary of some oxido-reductive reactions although the possibility of different points of attack resulting in the same effect, i e in the prevention of hepatic necrosis cannot be excluded.

Necrosis of the liver is in most cases demonstrable in the gross. In the few instances when it stays latent it may be brought out in a very ingenious way as devised by Professor R Kuhn, Heidelberg (personal communication). Normal livers of freshly killed animals immersed in a 0.5% aqueous solution of phenyl tetrazolium chloride will take up a bright red color (formazan formation). In contrast necrotic livers or necrotic foci will retain their normal color after exposure to the dye. Maybe this method which is based on oxido

reductive reactions will be of value in testing specimens of liver obtained through biopsy

Watson Is it an external stain?

Gyorgy Yes

Hanger The non injured cells take up the stain?

Gyorgy That is correct

Finally I cannot resist from calling attention to some practical implications of the research on hepatic necrosis, which appears to me rather intriguing especially because for me as a pediatrician, it has pediatric connotations

In Rh incompatibility several research workers, among them Sir Leonard Parsons (Birmingham, England), N Philpott (Montreal) and myself have discussed the role of liver injury as a central patho genetic factor in the outcome of the disease, including especially the hemorrhagic manifestations and also kernicterus Hepatic injury fol lows some until yet unidentified phases of the antigen antibody reac tion, which in itself occurs obviously as the first chain in the events linked with the syndrome of Rh incompatibility The hepatic injury is characterized chiefly by acute zonal or massive necrosis Protection of the liver may be attempted by methionine Philpott has already presented preliminary evidence in favor of this view We may now add the further possibility that during the sensitization process which is at the very bottom of Rh incompatibility, the normally low vitamin E stores of the fetus and the newborn are further depleted Hepatic necrosis is one of the characteristic sequelae of vitamin E deficiency under particular conditions Edema and pulmonary hemorrhage are often encountered in very severe human erythroblastosis and seen also in experimental vitamin E deficiency in animals Studies are in progress jointly with Dr Carl Bachman, Professor of Obstetrics, School of Medicine, University of Pennsylvania, on the combined use of methionine and vitamin E in the prevention of the unspecific sequelae of Rh incompatibility, such as hepatic necrosis, kernicterus, pulmonary hemorrhage and edema It is *not* expected that the anemia, based on immunological antigen antibody reaction, will be influenced by the administration of methionine and vitamin E

In summary, I would like to conclude that it is possible to produce regularly by purely dietary means, massive necrosis of the liver in rats Further, it is possible to prevent such massive necrosis of the liver again with regularity, by supplements of sulfur containing amino acids or of vitamin E The final cause and pathogenesis of massive necrosis has still to be elucidated

Best I have a question along the same line You say 'by dietary

means you produce necrosis, and yet you really favor now the presence of a toxic substance Those are not compatible in my opinion because you can produce almost anything by dietary means if you add some toxic substance

Gyorgy The question of deficiency versus intoxication is difficult to answer If the experimental diet does not contain a poison as we understand poison and consists only of the usual food constituents such as casein yeast and fat as employed in nutritional research conditions produced by such ration could be considered of dietary origin If we would add to the ration butter yellow, we added a poison

Best This condition occurs if you use a certain yeast but not with another yeast

Gyorgy Our experimental yeast is used in England for baking purposes

Fremont Smith May I suggest putting quotation marks around dietary? You would not object?

Gyorgy No

Fremont Smith That would satisfy Dr Best?

Best Yes for the time being

Gyorgy If one adds raw egg white to the diet biotin will be bound and inactivated in the intestine Thus the egg white will appear as a toxic factor leading however to a deficiency condition

Best I accept that as dietary

Gyorgy On a diet low in casein one may get necrosis in 40% of the experimental animals There is the possibility that on such diet intermediary toxic metabolites are not detoxified through lack of sulfur containing amino acids or vitamin E It is a fair assumption that toxic metabolites are continuously produced in our body which require detoxication

Watson Could you not classify them as absolutely and relatively toxic? Arsenic is absolutely toxic and egg white only relatively poisonous

Best But the mechanism is different

Gyorgy One may go even further At the end of the last and at the beginning of the present century so-called autointoxication from the intestine was a much discussed subject in medicine Such intestinal poisons are metabolites or products of intestinal bacteria and special food constituents per se innocuous may promote their production in the intestine

Best Dr Schwarz would you tell us about some of your studies?

Schwarz It is both an honor and a pleasure for me to participate in this conference and I thank you for this opportunity

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Best Dr Schwarz would you tell us about some of your studies?

Schwarz It is both an honor and a pleasure for me to participate in this conference and I thank you for this opportunity

The experiments on which I will base my discussion were conducted at the Kaiser Wilhelm Institute at Heidelberg between 1940 and 1945 Due to general events, complete publication of my research has been delayed However two papers appeared in 1944, one of which described the production of liver degeneration by dietary means, and the occurrence of liver protecting substances (K Schwarz, Ztschr physiol chem *281*, 101 (1944)) while the second described the concentration and identification of vitamin E as a liver protecting factor (Ibid, *281*, 109 (1944)) Unfortunately, these papers did not become available in this country until 1946 Other details have appeared subsequently (Ibid *283* 106 (1948), Ibid *283*, *186* (1949))

I would like first to discuss Dr Gyorgy s experiments in relation to ours I was very much pleased to hear that Dr Gyorgy has been able to confirm our results with vitamin E

With reference to the points where our results disagree I think there is much less discrepancy than Dr Gyorgy supposes Dr Gyorgy mentioned that sulfur containing amino acids had no protective effect in my experiments This is true only in part, namely in regard to the so called casein VI liver injury

As I pointed out at the International Conference on Vitamin E (K Schwarz, Ann N Y Acad Sciences *52* (1949)) and is evident from publications, I had to distinguish between three different types of dietary liver injuries These are different in their histological and pathological aspects and in their relation to different liver protecting agents The damage which is produced by yeast as a main protein source in synthetic diets is the one applied by Dr Gyorgy In my experiments cystine and methionine had exactly the same protective action as in Dr Gyorgy s tests when studying yeast liver damage (K Schwarz, Ztschr physiol chem *283* 186 (1948))

In his experiments Dr Gyorgy has been unable to reproduce the casein VI liver damage This might be due to technical differences

1 He used casein as a starting material which was not as highly purified as necessary It can be shown easily that crude casein has a protecting action against liver degeneration although the factor in or property of casein which is responsible for this action has not been identified If we assume — as seems probable — that this action is due to a protective substance, then it must be rather firmly bound to the protein since it is so difficult to remove The material used in our laboratory for the production of casein VI had been thrice reprecipitated from alkaline solution using the method of Hammersten and had been extracted with ether before the preparation of casein VI was started

2 When he tried to produce the casein VI liver damage, Dr Gyorgy used a material named Vream instead of extracted butter fat (P Gyorgy and H J Goldblatt J Exp Med 89, 245 (1949)) The method of preparation of butter fat has been given in detail together with that of casein VI in the first of our publications Vream (Swift & Company) is a shortening of vegetable origin In Dr Gyorgy s experiments, liver damage was inhibited by another vegetable shortening, namely Crisco (P Gyorgy, Sixth Conf on Liver Injury Josiah Macy, Jr Foundation, N Y (1947)), and it seems quite possible that Vream might have the same protective quality as Crisco

In our experiments we watched not only the purity of the casein and fat, but we also used a specially refined sucrose which was tested microbiologically to be free of growth promoting factors

3 In my experiments it was necessary to prevent the animals from getting too many protective substances during their nursing period if liver injury was to be obtained Therefore the breeding mothers were put on cooked rice and skimmed milk starting from the date of birth of their litter The young animals were put on experimental diets as soon as they weighed 28 to 30 grams The liver damage did not appear as early or as regularly when the mothers received stock diets or when the young animals were allowed to remain for a longer time with their mothers Several hundred days were needed to develop the liver damage when adult rats were put on experimental diets

4 Furthermore it is natural that differences occur among animals of different strains or litters In our experiments the rats were not inbred but the litters were always evenly distributed over the experimental and control groups

In order to give a more complete picture I shall outline the development of these experiments Between 1937 1939 efforts were made in Heidelberg to isolate the so called filtrate factor using lactobacillus plantarium (streptobacterium plantarium Orla Jensen 10S) as a test organism The main component of this fraction finally turned out to be identical to pantothenic acid (E F Moller Ztschr physiol chem 260 246 (1939)) In highly purified concentrates of this principle, prepared from rice bran I was able to separate a new growth promoting factor which was necessary for our lactobacillus (E F Moller and K Schwarz Ber Dtsch Chem Ges 74, 1612 (1941)) The new factor was called H' we were finally able in 1941 to isolate the factor H' and identify it as p amino benzoic acid (R Kuhn and K Schwarz Ibid 74 1617 (1941))

Before we knew that factor H' was p amino benzoic acid, experiments were started to determine whether it was a vitamin for the

rat During these experiments I happened to develop a method to produce fatal liver degeneration in young rats by dietary means I tested the p amino benzoic acid content of a variety of caseins and found that a large amount of this factor was present As much or even more of this material could be found in crude casein as in the best natural sources, liver or yeast The content of p amino benzoic acid in casein preparations seems to be a good method to determine the purity of the material Even so called vitamin free and highly extracted samples of casein contain an amount of p amino benzoic acid which is easily detected by the growth test with lactobacillus plantarium P aminobenzoic acid seems to be firmly bound to the protein molecule and it is necessary to heat the casein under slightly alkaline conditions in order to eliminate factor H' In our procedure the casein was boiled in solution starting with pH 8 7 for three hours During the treatment the pH decreased slowly to 7 5 The substance which resulted after reprecipitation with acid, drying with alcohol and extraction with ether was named casein VI

When young rats were fed on diets with this highly purified casein VI, more than 84 percent of them died within 12 to 80 days (average 42 days), in a quickly developing coma leading to death in a few hours Their livers were severely degenerated The injury appeared so suddenly that it was almost impossible to detect it before the onset The development of this disease was not related to p amino benzoic acid A daily dose of 1 milligram had no influence on the casein VI injury The content of this factor thus was used merely as titer for the purity of the casein

The real reason for the fact that casein VI induces liver degeneration, whereas the starting material does not, is not yet known Casein VI has the same optical rotation and nearly the same C, H, N, P, and S content as the Hammersten casein from which it was prepared Casein VI itself did not seem to be toxic By varying the casein VI content of the diets, it could be shown that the incidence of liver degeneration fell with rising amounts of casein VI Apparently, the sulfur containing amino acids were not involved as causative factors in casein VI damage as they are when liver injury is developed on yeast diets, since the addition of cystine or methionine to the diet had no protective effect The methionine content of casein VI is practically the same as in the starting material The cystine content of casein is apparently too small to play an important role, nevertheless, we considered the possibility that the small amount of cystine present (0 4%) could be transformed into lanthionine during the alkaline treatment The addition of synthetic lanthionine to a diet with purified, but not liver injuring casein did not induce liver degeneration

Taking all these points into consideration it seemed likely that no toxic agent was produced by the alkaline treatment but that a liver protecting factor was removed

We were able to produce the casein VI liver injury as a matter of routine but a considerable variation occurred in the incidence of liver degeneration and in the time of survival in 35 experiments between 1941 and 1943 Regularly in September and October liver degeneration occurred in a smaller percentage of the animals and only after a longer time

It has been mentioned as first stated by F S Daft W H Sebrell and R D Lillie (Proc Soc Exp Biol & Med 50 1 (1942)) (W H Sebrell Sixth Conf on Liver Injury Josiah Macy Jr Foundation p 71 (1947)) that liver degeneration has to be sharply separated from dietary liver cirrhosis In over 1300 animals dying from acute liver degeneration in our experiments not one was found to have a definite liver cirrhosis All our animals were given 1 milligram of chol ne chloride daily This was sufficient to protect against kidney hemor rhage Larger daily amounts of choline (20 mg) did not influence the development of the casein VI injury

We used casein VI liver damage between 1941 1943 as a method of routine to test the protecting activity of a large number of dif ferent materials and concentrates Several agents were found in nature which protected against the development of this type of liver degeneration

Wheat germ was especially active in our prophylactic tests Using this as a starting material effects were made during 1941 1943 to isolate the active principle contained in this material The final con centrates contained 44 percent of a substance which was shown to be vitamin E I then found that synthetic dl alpha tocopherol acetate was active This was a surprising result because our animals had received a certain amount of vitamin E namely 50 mg per week as a matter of routine during all these experiments This was enough to protect against damage to the reproductive organs using normally purified casein but 17 or 18 times as much vitamin E was needed to protect against the development of liver damage in casein VI d ets

After discovering the protective action of vitamin E upon the liver under the conditions of our dietary experiments I attempted to de termine whether other liver damages could also be influenced by vitamin E In the German veterinary literature an acute liver dystrophy has been described several times which arose in baby pigs when high levels of cod liver oil were fed (W Nikolaus Tierarztliche Rund schau 43 1 (1937) W Nikolaus Arch Tierheilkde 73 428 (1938) Tiedge Dtsch Tierarztl Wochenschr 45 132 (1937)) Incorpora

tion of 10 or 20 percent of cod *liver oil into the scheme* of our experimental diets — with highly purified, but not alkaline treated casein — caused death of young rats after several weeks with marked liver damage There were *gross and microscopic pathological differences* between the casein VI damage and the cod liver oil damage Five milligram percent of dl alpha tocopherol acetate in the diet fully protected the animals against this disease

The third type of liver damage, the so called yeast degeneration, was different in its origin as well as in its manifestations The injury *occurred if large amounts of yeast* were fed as the main protein source in synthetic diets This damage has been considered for a long time to be due only to the lack of cystine and methionine in yeast protein *I found that vitamin E was quantitatively inhibiting*

In summarized form the three types of liver damage can be defined and differentiated as follows

1 *Casein VI Damage*—This injury is produced by the incorporation of highly purified casein VI into synthetic diets The animals are in good physical condition and grow fairly well for several weeks They suddenly develop a peculiar coma, respiration becomes slower and deeper, the heart beat slackens, the body becomes cold and death ensues in a few hours The pathological picture is that of an acute liver degeneration The massive necrosis is localized in the central part of the lobules or at least starts there Hemorrhages in the liver are seen in almost every case Sometimes the major part of the organ is infiltrated with blood The degenerated parts show mostly a moderate fat infiltration Ceroid pigment but no fat is found in the endothelium of the capillaries * Casein VI injury is not influenced by feeding sulfur containing amino acids The damage can be quantitatively inhibited by vitamin E A daily dose of about 130γ of dl alpha tocopherol acetate is sufficient to protect most of the animals Five milligram percent of this substance in diets was protecting in every case Xanthine, which was found primarily to be active against chloroform damage (J C Forbes and J S McConnell, Proc Soc Exp Biol & Med *36*, 359 (1937), R C Neale and H C Winter, *J Pharmacol Exp Therapeut*, *62*, 127 (1938), H N Barett, D L McLean and E W McHenry Ibid *64* 131 (1938), J C Forbes, Ibid *65*, 287 (1939)), had a protective *action The probability that there exists* another protecting factor in crude casein has been outlined above I might mention that whey also has an inhibiting effect

* The histological examinations have been carried out by Prof Dr Dobberstein Tierarztliche Hochschule Berlin and Doz Dr Velten Path Institut University of Heidelberg

2 *Cod Liver Oil Damage*—This injury is induced by diets containing normally purified casein and 10 to 20 percent of cod liver oil The animals die after several weeks The gross appearance as well as the histological picture of the livers is different from the casein VI damage No hemorrhages occur The livers are highly infiltrated with fat Microscopic examination reveals in many but not in all cases a degeneration which is evenly distributed over the entire lobule The severe fatty infiltration exists in sharp contrast to the normal body fat depots which have disappeared almost completely The voluntary muscles of these animals are mostly degenerated Muscle degeneration is a well known finding in vitamin E deficiency The kidneys show a zone of calcification in the intercorticomedullary region This is due to the hypervitaminosis D Cod liver oil damage to the liver and muscles is inhibited quantitatively by vitamin E The influence of vitamin E in this case might be explained by its well known action as an antioxidant It was first demonstrated by C G Mackenzie J B Mackenzie and E V McCollum (Science 94 216 (1941)) by the work of H Dam (Proc Soc Exp Biol & Med 52 285 (1943) J Mount Sinai Hospital 12 1021 (1946)) and by Mason and his group (L F Filer Rumery and K E Mason First Conf on Biol Antioxidants Josiah Macy Jr Foundation (1946)) that certain features of cod liver oil intoxication have much in common with vitamin E deficiency symptoms and that this vitamin antagonizes the overburdening of the body with cod liver oil It is not yet known if other substances for example cystine methionine or xanthine are effective

3 *Yeast damage* (rat eclampsia)—The injury which is caused by the administration of comparatively large amounts of yeast as the source of amino acids in purified diets was first observed by A Hock and H Fink (Zschr Z physiol chem 278 136 (1943) Ibid 279 187 (1944)) and by H P Himsworth and L E Glynn (Clin Sci 5 93 (1944) Ibid 5 133 (1944)) My experiments on this subject have been carried out between 1942 and 1945 Young rats on diets containing 24% yeast corresponding to 12% protein and 3% casein* died after several weeks with symptoms which were similar to those in the other liver damage experiments They lapsed into a coma followed in a few hours by death However one remarkable difference could be observed In a certain percentage convulsions could be seen during the coma The condition usually began at night so that many of the animals were found dead in the morning without being observed The livers of animals dying of the yeast injury were very similar macroscopically to those with casein VI damage Yel- [illegible]

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* compos on yeas 24 ase n 3 butte fat (extracted) 4, sucrose 63 8, salt mix (Add onal am n see K S hwa z Z s h physiol chem 281 103 (1944)) [illegible]

areas of degeneration and other parts with hemorrhages were irregularly distributed over the organ Normal tissue could hardly be found The color of the degenerated regions had a more pink tinge compared *with the casein VI damage Histologically* there existed a definite difference The degeneration in the casein VI injury is central, whereas the yeast injury begins in the periphery of the lobule A well developed fat infiltration is found not only in the liver cells themselves but also in star cells The kidneys of these animals show an alteration which has been defined by J Dobberstein and A Hock (Ztschr physiol chem *280*, 21 (1943)) as glomerulonephrosis These authors pointed out that the entire picture corresponds closely to the changes which can be found in human cases of eclampsia of pregnancy The term 'rat eclampsia' has been proposed for this type of dietary damage in the rat This proposition has been criticized by obstetricians who want to reserve the term for their own field regardless of the fact that eclampsia means any convulsive seizure of peripheral origin The term yeast injury is rather unsatisfactory because the same liver injury, or at least a very similar one, can be produced by several other dietary methods, i e by diets without *yeast* but with a similarly low content of cystine and methionine Diets with 10% or less casein cause liver damage Most of the classic experiments of Gyorgy and Goldblatt about liver injury were accomplished with this type of ration (P Gyorgy and H Goldblatt, J Exp Med *75*, 355 (1942)) The regimen with 10% or less casein enabled these authors first to produce liver damage (necrosis and cirrhosis) regularly, and though necrosis and cirrhosis were not as clearly separated at this time it is quite obvious from their *early* work that *yeast* (of *American* origin) inhibits the development of necrosis The question, however, of whether this low casein damage is in all respects identical to the yeast injury — induced by European yeast — remains to be thoroughly investigated A Hock and H Fink (Ztschr Naturwiss *2b* 203 (1947)) described several other diets containing 8 to 15 percent of protein in the form of potato protein, pea protein, cereal protein, and gelatin in various combinations which induced liver degeneration I should mention that these experiments were started as a result of discussion remarks of mine made in 1944 to the effect that we should consider the possibility that yeast might contain a substance with toxic effects The liver degenerations obtained by Hock and Fink could all be inhibited or at least beneficially influenced by cystine I think the fact that these damages can be produced by a variety of diets without yeast but poor in cystine and methionine, is an argument against the theory that yeast itself contains a toxic principle

Yeast injury (rat eclampsia) was for a long time thought to be due

only to the defect of sulphur containing amino acids in the diet (H P Himsworth and O. Lindan, Nature *163*, 30 (1949)) I found however that 5 milligram percent of dl alpha tocopherol acetate gave full protection against this damage in the same way as against the other types of liver damage described above * Rat eclampsia therefore cannot be considered to be due to a simple lack of sulfur-containing amino acids It is a disease caused by the simultaneous absence of vitamin E on the one side and cystine methionine on the other Each of these substances alone is able to prevent the damage It is an injury which can arise only if two diverse, important, dietary components are insufficiently supplied at the same time The term "ambogene" deficiency has been used to define this type of injury (K Schwarz, Ztschr physiol chem *283*, 186 (1948)), which lately has become of growing interest in nutritional research The phenomenon of an ambogene disease seems to indicate the existence of a close metabolic connection between the substances involved In our case the metabolic relation between vitamin E and sulfur containing amino acids is not yet known We find that one molecule of tocopherol has the same protective effect as 200 400 molecules of methionine or cystine This ratio might perhaps signify a catalyst substrate proportion

The finding that — at least some — American yeasts do not as easily induce liver injury in synthetic diets as European varieties is of considerable interest Dried yeast has been used more and more as a common foodstuff during the last few years in Europe In 1943 and 1944, we compared several German yeasts of different kinds and origins A quantitative difference but no fundamental distinction was found among brewers yeast (Loewenbraeu Muenchen), torula yeast grown on sulfit liquor (Zellstoff Fabrik Waldhof), and torula cultivated on a mixture of molasses and wood hydrolysate (Bergin A G Mannheim) All these induced liver degeneration, the animals from brewers yeast survived on the average longer and the incidence of liver necrosis was smaller in this group

We were unable to separate a toxic substance from yeast by thoroughly extracting with boiling water or fat solvents About 40% of the dry weight of yeast can be brought into solution by these means The residue of the extraction consists mostly of yeast protein When this residue was employed instead of yeast, liver degeneration resulted to the same extent as with the untreated material The explanation for the quantitative differences among various yeasts, especially among European and American ones, might perhaps be found in the amount of sulfur containing amino acids present Most American yeasts are grown in a much richer medium than were the yeasts produced in

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Presented at the meeting on Protein July 1944 in the Plassenburg Kulmbach

Europe during the war The cystine and methionine content of American yeasts seems to be significantly higher In European materials cystine sometimes could not be detected at all This might be partly due to technical difficulties The exact determination of small amounts of cystine in material like yeast is problematic because cystine reacts during hydrolysis with carbohydrates and thus is destroyed It is obvious, however, that there is a definite difference between the amount of cystine and methionine present in American yeast and European yeast

The content of organic sulfur in yeast is comparatively high It is sometimes higher than in casein, for example, and if casein is replaced by yeast protein in synthetic diets the animals get more organic sulfur but nevertheless develop liver damage which can be prevented by sulfur containing amino acids This could support the toxicity theory if we assume that the organic sulfur in yeast is present mainly in the form of cystine and methionine However, the quantitative analysis of German yeast for methionine and cystine gave values which generally did not correspond to the total amount of organic sulfur present In some instances the deficit was as high as approximately 55 per cent of the total organic sulfur Therefore it seems possible that yeast contains a remarkable amount of organic bound sulfur which is not methionine or cystine The content of thiamine biotin, and adenyl thiomethylpentose in yeast is too small to explain more than but a few percent of this deficit We investigated the quantity of ergothioneine in yeast and found no remarkable amount of this substance

The question of toxic factors the toxicosis theory , has been discussed during the development of many biochemical problems especially those of medical application This was the case, for example, with the thyroid function, with the origin of pernicious anemia etc As far as different dietary liver injuries are concerned it can be stated that at least some of them, namely the low casein damage and the injuries caused by diets containing potato protein and gelatin etc are obviously not induced by special toxic agents We know on the other hand two forms of liver damage which are apparently caused by the presence of dietary constituents with deleterious effects the cod liver oil damage and the liver degeneration produced by the administration of large amounts of cystine in rations This injury, induced by 5 or more percent of cystine in synthetic diets was the first dietary liver injury described (A C Curtis and L H Neuburgh Arch Int Med 29 828 (1927) M X Sullivan W C Hess and W H Sebrell Pub Health Rep U S P H S 47 75 (1935) R D Lillie Ibid 47 83 (1935) D P Earle and J Victor J Exp Med 73 161 (1941))

We must assume that cystine plays an important part in some metabolic reaction of the liver and that this function is so fundamental

that the liver cell degenerates if the reaction fails In view of this it is not difficult to reconcile the two facts that on the one hand a small amount of cystine protects against certain liver degenerations while on the other hand a large amount of this substance causes the same The metabolic reactions in which cystine is involved are enzyme reactions and cystine is the substrate It is quite a common and well known feature of many enzymes that they are inhibited by a surplus of substrate This would only be one of different possible ways to understand the phenomenon

In metabolism we always find a certain range within which every normal nutrient is harmless Even sugar is injurious if this margin is exceeded The tolerance for normal dietary constituents often is remarkably displaced in deficiency diseases If for example a rat is vitamin B_1 deficient a relatively small dose of sugar has a fatal effect

It is debatable whether we should apply the terms toxic , toxine , and intoxication in this case just as it seems questionable whether we should use them with the cod liver oil injury or the effect of large doses of cystine Perhaps it is better to define and classify these conditions simply as dietary stress especially if we have no proof — as is the case with the yeast injury and the casein VI damage — whether a toxic component really is involved or not the more so since the term intoxication has many well established pharmacological and metabolic implications These might not be in accord with the characteristic picture of metabolic pressure and final breakdown of cell functions as we see in the different forms of dietary liver degeneration *

DISCUSSION

Best May we have a general discussion at this point?

Gyorgy I would like to make a few comments First of all I do not wish to give the impression that I assume a direct toxic factor in yeast I did not say there was one What I said was that the yeast might lead to the production of a toxic factor Where it originates I don t know but most probably through the interaction of the intestinal flora

As previously stated I do not have the analytical figures for cystine and methionine in the British yeast However there are several discrepancies in the observations which make it difficult to explain the necrogenic effect of British yeast compared with the American yeast by the lower content of British yeast in sulfur-containing amino acids.

* In accord with the wishes of the Chairman and the participants of the Conference Dr Schwarz has prepared his report in greater detail than he was able to present at the time of the meeting Consequently some of his data was not available for comment or discussion by the group —Editor

Be that as it may, I do not want to postulate the presence of a direct toxic substance in yeast

Schwarz I think it is very difficult to differentiate between intoxication and the lack of an important substance in the organ, especially of a substance which is working catalytically If such a substance is lacking, then you will have, perhaps, an intoxication of something which is not changed because it is lacking The most famous example of that is vitamin B_1 and pure pyruvic acid

Best You might have an enzyme system intoxicated by a poison and the same enzyme system completely inactivated by the lack of a vital factor It would be the same mechanism

Schwarz That is what I wanted to say

Patek From the remarks of Dr Gyorgy it seems that the old concept of autointoxication is not so fanciful I wonder whether it would be feasible to feed some animals a substance which would inhibit bacterial flora, to see if this would influence the production of necrosis

Gyorgy In cooperation with Dr Joseph Stokes, Jr we are studying at present the influence of aureomycin, mainly as an intestinal antiseptic, given by mouth, in a group of rats receiving the severely necrogenic ration containing the British yeast, compared with a control group receiving the same diet without aureomycin The results will be forthcoming during the next three months *

Best Dr Hoffbauer do you have any comments on this subject?

Hoffbauer What I have to say will not require much time At the conference last January, Dr Best raised the question as to whether or not anyone had studied the porphyrin excretion in animals in which dietary liver injury was produced In our laboratory we have set about to do that As one aspect we were anxious to study animals in whom massive hepatic necrosis was produced Dr Gyorgy was kind enough to show me how to set about this problem and provided me with a supply of yeast from the United Yeast Company in Great Britain Since January we have observed the production of massive hepatic necrosis in 14 of 22 rats which were started on the diet This occurred within 35 to 80 days Other animals which we have sacrificed at intervals have not shown the lesion A few still remain alive Estimations of urinary and fecal coproporphyrin have been carried out by Dr Greenberg The estimations have been made at varying intervals We had hoped that we might be able to predict the onset of massive hepatic necrosis by an increase in the excretion of this pigment coproporphyrin which can be readily recognized by its fluorescent qualities

* Added to the proof Aureomycin exerted a significant protective or at least a delaying effect on the development of this type of hepatic necro[illegible]

In the paper reported by L. E Glynn, H P Himsworth and A Neuberger (Brit J Exp Path 26, 326 (1945)) mention is made that Professor Rimington studied a few samples of urine from similar rats and failed to find an increase in coproporphyrin More recently C E Dent and C Rimington (Biochem J 41, 253 (1947)) reported that animals maintained on alkaline treated casein diet showed an increase of coproporphyrin We have not found, in any animal, a significant increase in either the urinary or fecal coproporphyrin These observations have not been carried out continually but in various times during the course of the experiment In two animals we were able to obtain urine specimens for the two days immediately preceding death There was no significant increase in these animals We have definitely encountered lesions which are identical with what Dr Gyorgy has shown you

We feel reasonably certain that, in the group of rats on the diet containing British yeast as the sole source of protein, massive necrosis of the liver has developed and yet there has been no significant increase in the urinary coproporphyrin excretion That may possibly be taken as some indirect evidence that we are not dealing with the usual type of chemical poisoning The common hepatotoxins usually result in an increase in the porphyrin excretion in the experimental animal

Gyorgy This has been demonstrated in rats?

Watson With sulfanilamide, by C Rimington and A W Hemmings (Lancet 1, 770 (1938))

Gyorgy That does not produce any necrosis as chloroform and carbon tetrachloride

Hoffbauer Carbon tetrachloride, if not given in such massive doses that it damages the kidney appears to cause an increase in urinary coproporphyrin excretion We must also keep the factor of renal damage in mind in urinary pigment studies If the kidney is damaged the material may not appear even though it should be expected to on the basis of liver injury I believe the rat will respond to some of the porphyrinogenic materials by an increase in coproporphyrin in the urine

Gyorgy Is there not a very acute onset of this type of injury? Himsworth has noted this and I can confirm it The rats are running around and then suddenly they are dead

Hoffbauer It has been amazing to us As I perhaps mentioned this afternoon one of the animals we had just removed from the metabolism cage used to collect the specimens seemed perfectly normal in all respects I was quite astounded to have the man in the laboratory call me up two hours later and say that the animal was dead I had no premonition that it was going to die

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Watson I think that perhaps fits with the fact that you have not seen any increase in porphyrin at all even in the 48 hours before death If that liver had been appreciably damaged, I think you would have found some increase It seems to me this must happen very abruptly I think the well being of the animal and lack of increase of porphyrin at least strongly suggests that

Best The liver damage does not kill the rat Something else may be acting quite independently

Watson I have asked Dr Hoffbauer to find out what the blood sugar is

Gyorgy Nobody has done it

Best You are not going to be able to do it You have extensive liver damage obviously

Schwarz I might mention that it may perhaps be a very quickly developing intoxication I have been able to transfer the coma of the dying rat to mice by taking the blood of the animals that were just going to die I could make the mice die This was done with the animals of the casein VI group and the yeast group I did not try it with the cod liver oil animals because I did not have an opportunity It is very difficult to get these animals because most of them die at night and the coma develops so quickly that you have only two or three hours of coma and then they are dead

I have tried to find liver damage before they died I killed them after one week, two weeks, and so on, and I did not find any liver damage I did find the liver damage only when the animals were going to die

Gyorgy The last two or three hours?

Schwarz That is quite true

Hoffbauer Did I understand you correctly that blood from these animals, just at the time of death, was lethal for mice?

Schwarz Not whole blood I prepared it

Hoffbauer You ground up the liver?

Schwarz I took only the blood and made a filtrate, I deproteinized the blood and injected this intraperitoneally into mice, very small mice, and they died

Armstrong What did you use as a protein precipitant?

Schwarz I prepared it by heating I did several other experiments, using I believe phosphotungstic acid, by reprecipitating with the phosphotungstic acid and by reprecipitating with sulfuric acid it was quite clear no other agent was in the filtrate

Armstrong It seems to me as I heard this discussion that this question

may be reminiscent of the unsaturated essential fatty acids antioxidant story

I should like to ask Dr Gyorgy if he has seen any evidence — he has told us about the liver — of scales on the tails Do the animals have hematuria? Is there any evidence of unsaturated fatty acid deficiency?

Gyorgy All have unsaturated fatty acids They all receive extra unsaturated fatty acid

Schwarz My animals did have linoleate

Gyorgy There were no tail changes, no kidney changes, no skin changes

Goldblatt I should like to ask Dr Schwarz one question and that is just what is he referring to when he speaks of three types of liver injury? Can you, Dr Schwarz, translate that in terms of morphology, or are you speaking of some kind of a functional liver injury?

Schwarz I would say that you can differentiate between these three types of liver injury histologically One, the casein VI injury, is more central, the other, the yeast injury, is more on the periphery The yeast type is connected with typical changes in the kidney The kidneys show 'glomerulonephrosis The whole syndrome is very similar to the syndrome which you can see in human eclampsia

The cod liver oil damage is distinguished by its heavy fatty infiltration of the liver cells, and is distributed homogeneously over the liver Thus, I believe one is able to differentiate all of these three types of damage

Best If you let these go on, does not the peripheral go all through the lobule, and the central if you follow it does it not become general as well?

Schwarz You can still differentiate it If you look over the liver, you may see several lobules uniformly involved but then you see others where the degeneration is just beginning and there you see it is beginning centrally or peripherally

Best Both Dr Gyorgy and I thought immediately of vitamin B_{12} It seems to be fairly obvious and we are anxious now to get a supply of alpha globulin from soybeans which is apparently completely free of vitamin B_{12}, because our diets have been full of this It is lipotropic It may spare choline

Schwarz There is a possibility that vitamin B_{12} may be involved That would give another factor which is involved in all these relations That might form an explanation, perhaps, for the differences between the several groups of authors too One is using crude casein and the other is using highly purified casein which is free of vitamin B_{12},

SECTION IV

RECENT FINDINGS CONCERNING THE ROLE OF THE LIVER AND KIDNEY IN CIRCULATORY HOMEOSTASIS[1,2]

Shorr Dr Knisely has made it abundantly clear from his studies on the liver, which he has just reported, that the regulation of the circulation in that organ is a very precise and extremely delicate process The glimpse he gave us of the structure and function of the vascular bed of the liver would indicate that its complicated vascular architecture and the subtle changes which take place in the behavior of the blood vessels were not haphazard and that they were undoubtedly related to the homeostatic regulation of the many functions which this organ serves

This concept of homeostasis has come to exert a major influence in our thinking about bodily processes The stability of the internal environment is secured through the action of three major organ systems, the kidneys, lungs and the capillary bed Without detracting from the importance of the first two, the final homeostatic adjustment, which maintains a stable internal environment for the tissues, can be attributed to the function of the capillary bed It is for this final stage in the homeostatic process that the function of the lungs and kidneys is in the last analysis conditioned

For that reason we have been particularly interested in studying the factors that regulate the function of the capillary bed Before discussing the role of the liver in this process it would be profitable to examine the present concepts as to the structure and function of this unit The original studies of Krogh have been recently extended by B W Zweifach R Chambers and their associates and the description that I am about to give is based on the observations of this latter group of investigators (*Am J Anat* 75 173 (1944)) Although the capillary bed may differ in some details from tissue to tissue, its basic structure appears to be fundamentally the same in all The architectural unit consists of a thoroughfare channel the metarteriole which provides a direct connection between the arterial and venous sides of the central vascular tree The metarteriole is discontinuously surrounded by smooth muscle cells which are most numerous in the proximal and absent in the distal portion which enters the venous channels All along its

1 Ephraim Shorr B W Zweifach R F Furchgott A Mazur S Baez and M A Payne Department of Medicine Cornell University Medical College and The New York Hospital

2 These studies were supported by grants from The Josiah Macy Jr Foundation, Eli Lilly and Company The Postley Hypertension Fund and The United States Public Health Service

length the metarterioles give rise to precapillary sphincters At the proximal end of the metarteriole these sphincters emerge at acute angles These angles become progressively less acute distally Each precapillary is surrounded by a well defined ring of smooth muscle cells which acts as a very competent sphincter The precapillary sphincters in turn give rise to the true endothelial capillaries through which fluid exchange takes place

The behavior of this architectural unit is characterized by periodic caliber changes of the metarterioles and precapillary sphincters This intermittent relaxation and constriction is termed vasomotion The fluid exchange through the true capillaries is profoundly influenced by this vasomotion through its effects in altering the ratio of hydrostatic to osmotic pressure These muscular vessels of the capillary bed also undergo fluctuations in their reactivity which can be conveniently measured by their responsiveness to the topical application of epinephrine

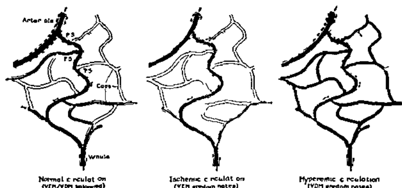


FIGURE 1 Diagrammatic representation of alterations in blood flow through capillary bed associated with predominance of VEM or VDM in the circulation Note ischemic circulation during VEM predominance resulting from constriction of precapillary sphincters with restriction of blood flow to main thoroughfare channel (the metarteriole) Note also the overall capillary circulation during VDM predominance as a result of relaxation of precapillary sphincters

I have illustrated in Figure 1 the three typical states of the capillary bed which result from alterations in vasomotion and reactivity under the influence of the vasotropic substances VEM and VDM with which we have been concerned In the normal state when neither predominates there is an intermittent flow through the capillary bed resulting from *the opening up of now one and now another precapillary sphincter* There is a continuous flow through the metarterioles If, however conditions are set up which lead to increased vasomotion and increased

scular hyperreactivity, this picture is profoundly altered Such a ondition is seen in the hyperreactive phase of hemorrhagic shock in ssociation with the appearance in blood of the renal vasoexcitor, VEM (E Shorr, B W Zweifach, and R F Furchgott, *Science* 102, 489 (1945)) Vasomotion is increased and the constrictor phase of precapillary sphincteric activity prolonged at the expense of the dilator phase As a result of this augmented precapillary sphincteric constriction, blood flow through the true capillaries is profoundly reduced, the major portion of the blood flowing through the metarteriolar or thoroughfare channels directly from the arterial to the venous side If, however, the hepatic vasodepressor, VDM, predominates, as during the hyporeactive stage of shock, vasomotion is depressed, the vessels become hyporeactive and quite another picture is seen The dilator phase of the precapillary vasomotion is now accentuated, the sphincters remain chiefly in the relaxed state and the capillary bed exhibits an overall flow which is in sharp contrast to the ischemic state previously described

We are now in a position to consider the consequences of the prevalence of each of these types of vascular behavior on fluid exchange within the capillary bed I have represented these different situations in very diagrammatic manner in Figure 2 In this figure, which shows

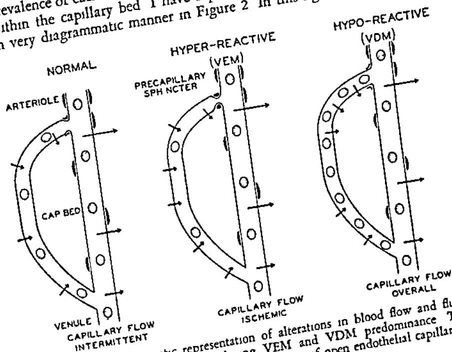


FIGURE 2 Diagrammatic representation of alterations in blood flow and fluid exchange in the capillary bed during VEM and VDM predominance The number of red blood cells represents the number of open endothelial capillaries The arrows represent the direction of the flow of fluid

a metarteriole, a precapillary sphincter and one endothelial capillary vessel, the number of open capillaries is indicated by the number of red blood cells which are drawn in the true capillaries, and the arrows indicate the movement of fluid in or out of the capillary bed Turning first to the normal state, we observe that the movement of fluid through the metarterioles is outward where the pressure is highest i e, at the proximal end, and the outward filtration decreases as the hydrostatic pressure is reduced by progressive resistance to the column of blood in its passage through this vessel In the true capillaries, hydrostatic pressure relationships are such as to make for outward filtration at the proximal end and for inward filtration at the distal end as the hydrostatic pressure is dissipated Attention should be called also to the action of the metarterioles, in favoring inward filtration at their distal ends This is a consequence of the wavelike progression of the smooth muscle contraction along the metarterioles The resultant rapid flow of blood through the metarterioles acts like a water pump to suck in fluid from surrounding tissues and tributary capillaries

When, as in hyperreactive shock, vasomotion is increased, and the precapillaries are largely in the constricted phase with a correspondingly reduced flow through the true capillaries, (as indicated by the smaller number of red blood cells in the diagram) all the factors making for inward filtration are favored The result is tissue ischemia, dehydration and hemodilution If, however the opposite condition of vascular hyporeactivity exists with sphincteric relaxation and an overall flow through the now open capillary bed then hydrostatic pressure predominates over a greater length of the capillary bed Outward filtration and edema formation are favored

It is on this portion of the vascular bed, which is so essential for the maintenance of a stable internal environment for the cells, that the hepatorenal principles with which our studies have been concerned, act Their action is ordinarily of a chronic nature It appears to consist in subtle adjustments to the fluctuating needs of the tissues When the integrity of the circulation is endangered i e, after blood loss when the balance is precarious, it is believed that these humoral agents can produce acute changes of a beneficial or a deleterious nature When they participate in chronic circulatory disturbances, the predominance of one or the other type of capillary behavior calls forth compensatory vascular adjustments on the part of the central vascular bed It is in this way we believe, that these humoral agents can bring about their long term effects on blood pressure When these adjustments on the part of the central vascular bed to the chronic type of behavior exhibited by the capillary bed fail to occur then circulatory homeostasis breaks down and situations such as edema formation and peripheral circulatory failure arise

These vasotropic principles which participate in the regulation of capillary function comprise a vasoexcitor, VEM, which has its origin in the kidney and chiefly in the cortical portion, and a vasodepressor, VDM, originating in liver, spleen and skeletal muscle The vascular effects of VDM from these different tissues are indistinguishable These principles are quantitated by their effects in enhancing or depressing the response of the terminal vascular bed of normal anesthetized rats to topically applied epinephrine In this test, which was devised by Zweifach and Chambers (B W Zweifach, *Methods in Medical Research,* edited by V R Potter, Year Book Publishers, Chicago, 1, *131* (1948)), the normal rat mesoappendix is exteriorized and the reactions of the vessels directly visualized before and after the injection of a test sample

The conditions have been ascertained for their formation *in vitro* and under certain circumstances *in vivo In vitro* experiments have shown that both VEM and VDM are normally formed by their tissues of origin only under anaerobic conditions They fail to appear under aerobic conditions Furthermore, under aerobic conditions normal liver inactivates VDM and normal kidney inactivates VEM

In addition to establishing their tissues of origin and the conditions under which these factors are formed and inactivated we have succeeded in isolating and identifying the liver vasodepressor VDM (A Mazur and E Shorr, *J Biol Chem 176* 771 *(1948)*) This has been found to be ferritin or apoferritin, the iron free moiety of ferritin Ferritin is an interesting iron protein complex first described by Laufberger in 1937 and subsequently studied by Granick and Michaelis in relation to its function in iron storage and transport It is a protein of a molecular weight of about 450 000 and exists in two forms i e, with and without iron The iron is not present as a porphyrin, but as ferric hydroxide The amount of iron is large constituting as much as 20% of the molecule by weight It is tightly bound within the molecule and can only be removed by strong reducing agents Both crystalline ferritin and apoferritin have similar and equal vasodepressor effects Hence, the vasodepressor action would seem to be related to the protein moiety rather than to the iron Profound vasodepressor effects are induced in the test rat weighing 100 125 gms with either substance in amounts of 0 0005 gamma of nitrogen Purification of VEM has not proceeded to the same extent Preparations have been obtained which are active in the rat test in amounts of approximately 0 0004 gamma of nitrogen It would appear to be a protein of relatively low molecular weight

We have followed the appearance of these principles in a variety of pathological circulatory states with results which I shall now describe

The first major circulatory disturbance which was studied in relation to these hepatorenal factors was experimental hemorrhagic and traumatic shock. Our studies were stimulated by the observation of Zweifach, Chambers and their associates, using vascular reactions of the exteriorized omentum and mesentery as an index, that the shock syndrome, in anesthetized animals subjected to prolonged hemorrhagic hypotension, proceeds in two stages an initial compensatory and a subsequent decompensatory stage (B W Zweifach, R E Lee, C Hyman, and R. Chambers *Ann Surg 120* 232 (1944)) The initial compensatory phase was characterized by a hyperreactive condition of the terminal vascular bed. This hyperreactivity is manifested by an increase in the intermittent constrictor activity (vasomotion) of the metarterioles and precapillaries The capillary ischemia which results from the accentuation of the constrictor phase of vasomotion and the consequent restriction of blood flow in the capillary bed to the thoroughfare channels the metarterioles serve to maintain an active venous return of blood from the tissues despite a reduced blood volume The ability to respond satisfactorily to transfusion seems to depend upon the maintenance of this type of peripheral vascular activity However, with the onset and prolongation of more drastic hypotension, vascular changes gradually appear in the splanchnic areas visualized which are antagonistic to the compensatory vascular adjustments prevailing in the initial stage and which eventually disrupt the peripheral circulation During the decompensatory stage there is a progressive reduction of epinephrine reactivity and a slowing and finally complete suspension of spontaneous vasomotion in the metarterioles and precapillaries with a resultant relaxation of precapillary sphincters The predominance of decompensatory influences on the peripheral circulation results in an increasing diversion of blood into the capillary side channels from which it is inefficiently returned to the active circulation because of the reduced blood pressure Once this decompensatory hyporeactive phase has fully developed a state of irreversibility or failure to respond to transfusions is reached It was further shown that blood borne factors are responsible in large measure for these vascular episodes by the passive transference of corresponding effects to similar vessels in the meso appendix of normal rats following the intravenous injection of blood samples removed during the evolution of the shock syndrome (R. Chambers B W Zweifach B E Lowenstein and R E Lee *Proc Soc Exper Biol & Med 56* 127 (1944))

The studies in our laboratory were designed to explore the mechanisms responsible for the occurrence of these humoral principles during experimental shock and to set up appropriate conditions for revealing their sites of origin in specific tissues as well as the environmental and cellular factors concerned in their formation and inactivation This

required a correlation of *in vitro* and *in vivo* phenomena which was made possible by the rat mesoappendix test Experimental hemorrhagic or traumatic shock was induced in dogs and rabbits under pentobarbital anesthesia and the syndrome interrupted during the hyper- and hypo-reactive phases Tissues were removed, appropriately sliced as for microrespiration studies, and extracted with saline The saline extracts were then assayed for their vasotropic activity It was found that the vasoexcitor, VEM, which predominates in blood during the initial compensatory phase of shock had its origin in the kidney and chiefly in the cortex The vasodepressor, VDM, which predominates during the decompensatory hyporeactive phase was found to arise in the liver, skeletal muscle and spleen During hemorrhagic shock the major role is played by VDM of hepatic origin During traumatic shock there is in addition a very considerable muscle VDM component This latter circumstance is believed to be responsible in large measure for the lessened tolerance to the equivalent degrees of hypotension of animals in traumatic as compared with those in hemorrhagic shock

The mechanism responsible for the sequential appearance of these vasotropic factors appears to be the progressive tissue anoxia characteristic of shock The earlier appearance of VEM is ascribed to the rapidity with which the renal blood flow is curtailed following a reduction in blood volume Although during the initial phase of shock there is a parallel reduction in blood flow to the liver consequent on moderate hypotension, this does not appear to be sufficient to initiate anaerobic processes in this organ and no VDM formation takes place Apparently the blood flow to the liver is normally in excess of its oxidative requirements as shown by the fact that the blood flow can be restricted to the hepatic artery with a maintenance of oxidative conditions (F L Engel, H C Harrison, and C N H Long, *J Exper Med* 79, 9 (1944)) Further support for this concept is provided by the work of Van Slyke and associates (D D Van Slyke, *Ann New York Acad Sc* 49, 593 (1948)) and Zweifach, Hershey, et al (B W Zweifach, S G Hershey, E A Rovenstine, R E Lee, and R Chambers, *Surgery* 18, 48 (1945)) who observed no rise in blood uric acid or ammonia with hemorrhagic hypotension of the moderate degree which leads to the development of peripheral vascular hyperreactivity However, following the induction of a more drastic hypotension which results in vascular decompensatory hyporeactivity a more severe curtailment of blood flow occurs in both liver and kidney Kidney blood flow is virtually abolished and the release of VEM into the circulation thereby prevented The liver now becomes hypoxic and VDM formation is initiated to continue throughout the rest of the syndrome It is under these conditions of more drastic hypotension that Van Slyke et al , and Zweifach et al ,

observed a rise in blood uric acid, a constituent which is maintained at normal levels in the blood by oxidative processes in the liver

Once VDM predominance and vascular hyporeactivity have persisted from 90 120 minutes the animal becomes irreversible to transfusion The question arose as to the reason for the failure of transfusions sufficient to restore oxidative conditions, at least temporarily within the organism The explanation for this failure is believed to reside in the progressive impairment of the mechanism by means of which the normal liver oxidatively inactivates VDM Studies of the liver, removed at intervals during the progression of the shock syndrome, showed that the VDM inactivation mechanism remained intact during the hyperreactive or reversible stage However, during the hyporeactive stage there occurred a progressive deterioration to total loss of this hepatic inactivation mechanism (E Shorr, B W Zweifach, and R F Furchgott *Ann New York Acad Sc* 49 571 (1948)) As a result, VDM could not be destroyed on the restoration of oxidative conditions and indeed VDM formation actually took place under aerobic conditions *in vivo* (R F Furchgott, B W Zweifach, and E Shorr *Federation Proc* 8 201 (1949)) An additional factor of

in the VDM inactivation system could not be restored This is shown by the experiments of H A Frank A Seligman, and J Fine (*J Clin Investigation* 25 22 (1946)) who found that viviperfusion of the liver via the splanchnic vein was ineffective if the perfusions were begun after the hyporeactive state had developed

Other experiments have been carried out which have directly related the liver VDM mechanisms to the capacity of the animal to recover from traumatic procedures Shock was produced in rats by means of the Noble Collip drum (E Shorr B W Zweifach and R F Furchgott, *Science* 102 489 (1945) and E Shorr and B W Zweifach unpublished data) 650 rotations of the drum are lethal for about 85% of normal animals However it is possible to build up resistance by training Sublethal drummings are given and the number of rotations progressively increased In a short time the rats are able to tolerate 1000 rotations without fatality Experiments were carried out on the liver VDM mechanisms in normal and resistant rats There was a striking difference between the two groups the normal rats formed more liver VDM during 500 to 700 rotations than did the resistant rats after 800 revolutions Livers removed from normal rats after drumming were less effective in VDM inactivation than livers removed after drumming resistant rats It was further found that the livers

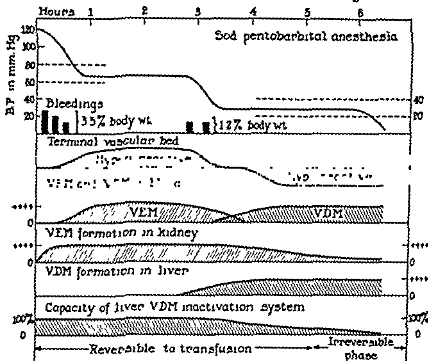


FIGURE 3

of the resistant rats were able in most cases to tolerate a period of anoxia of 2 hours *in vitro* and still preserve the capacity to inactivate VDM In contrast, livers from normal rats, like livers from normal dogs and rabbits, suffer profound deterioration of the VDM inactivation system after a similar period of anoxia (Fig 3)

From these and other correlative experiments a concept of shock has evolved which attributes a major role to these vasoactive principles of renal, hepatic, and skeletal muscle origins The initial reaction to hemorrhage, or extravasation of blood or fluid into traumatized limbs, leads to a reduction in blood volume and to adaptive vasoconstriction which involves splanchnic viscera as well as peripheral tissues The reduction in renal blood flow is profound and initiates the formation and release of VEM into the circulation This factor leads to the development of a specific kind of hyperreactivity in the terminal vascular bed which serves to restrict the flow in the true capillaries, send the blood through direct thoroughfare channels from the arterial to the venous side and thus maintain an effective circulation in the face of a reduced blood volume As long as VEM predominance and vascular

hyperreactivity prevail the animal is responsive to transfusions Should more drastic hypotension ensue, there is a further restriction of blood flow to the splanchnic viscera which leads to hepatic hypoxia VDM formation and its release into the general circulation VDM now overwhelms VEM whose further supply is shut off by the virtual abolition of renal blood flow As a result of the action of VDM hyperreactivity is replaced by peripheral vascular hyporeactivity of a decompensatory character The precapillary sphincters are relaxed there is an overall capillary blood flow and a progressive diversion of the reduced blood volume into an ever expanding capillary bed Through the local action of VDM upon the vessels of the splanchnic viscera

total loss of the hepatic VDM inactivation mechanism As a result the temporary restoration of oxidative conditions by transfusions is ineffective in liberating the vascular bed from the decompensatory effects of VDM Transfusions exert only temporary beneficial effect on blood pressure and then are lost from the effective circulation by their sequestration in the splanchnic viscera The potential capacity of these abdominal viscera to pool blood as a consequence of a local action of VDM on their vascular bed is believed to be quantitatively adequate to explain the ineffectiveness of blood replacement therapy at this stage

From these studies on shock we then turned to an exploration of the possibility that the hepatorenal factors with which we are concerned

experimental

120 (1948)

Tr A Am

Physicians 60 28 (1947)) We produced experimental renal hyper

clamp and assayed

thin a few minutes

was found in the

renal vein blood and within a few hours in the peripheral circulation It was observed to be present in blood throughout the period of acute hypertension but when the blood pressure leveled out at chronic hypertensive values the blood reverted to a neutral reaction At this point it seemed as if we were encountering a situation similar to that of renin which is present in measurable amounts in blood during the acute stage of renal hypertension but becomes undetectable when the chronic stage is reached However the development of procedures by which VEM and VDM could be fractionated in mixtures soon showed us that this was not the case (E Shorr and B W Zweifach *Federation Proc* 8 146 (1949)) I shall describe these procedures briefly I have

already pointed out that normal kidney cortex can inactivate VEM on aerobic incubation *in vitro* This property has been taken advantage of to wipe out VEM in mixtures of VEM and VDM Incubation of such a blood sample with slices of normal kidney for one hour in oxygen at 37 5°C serves to inactivate the VEM present and permit VDM to exert its effect unopposed in the test rat We have also prepared antisera to crystalline ferritin by its administration to rabbits (A Mazur and E Shorr, *Federation Proc* 8 228 (1949)) The incubation of a blood sample containing VEM and VDM with antiferritin serum inactivates VDM and unmasks VEM Utilizing these procedures for the assay of blood from renal hypertensive animals throughout the evolution of the syndrome, we found that the neutral reaction to which the blood reverted during the chronic stage of hypertension was due to the progressive release of VDM by the liver until its concentration in the blood was such as to neutralize the VEM which was being discharged by the kidney If one then follows the hypertensive animal for months and indeed years, during the chronic phase of the disease, one finds the invariable presence of both factors usually in a neutralizing ratio but occasionally with a temporary predominance of VEM or VDM In an occasional animal which has gone into the malignant phase we have observed a resumption of VEM predominance

It was now of interest to examine the mechanisms underlying the alterations in these humoral factors which followed partial constriction of the renal arteries The normal kidney never forms VEM under oxidative conditions but only during anaerobiosis The kidney in renal hypertension shows a very striking difference Within a few hours after the application of the Goldblatt clamp a metabolic alteration occurs as a result of which VEM is now formed under both aerobic and anaerobic conditions This inability to restrict VEM formation to anaerobiosis represents a critical metabolic defect which persists throughout the course of the disease Although its initiation probably is due to the renal hypoxia following partial constriction of the renal artery it is doubtful whether this explanation will hold for its persistence inasmuch as there is no good evidence of persistent renal hypoxia following circulatory adjustments to the renal artery constriction The oxygen consumption of these clamped kidneys *in vitro* may remain within the normal range for the dog

We have also studied renal hypertension in the rat and essential hypertension in man by the same methods In the rat the same defect appears in the kidney as was found in the dog and the humoral factors are present in similar fashion But we found as well a very specific alteration in the mesenteric vascular bed in renal hypertension in the rat (B W Zweifach S Rosenfeld and E Shorr *Federation Proc* 7,

139 (1948)) There is first of all an initial temporary increase in reactivity of the muscular vessels of the capillary bed as shown by an increased responsiveness to topical epinephrine This hyperreactivity is confined to the acute stage and disappears when the chronic phase is reached There is a parallel temporary increase in the sensitivity of the mesenteric vessels to the intravenous injection of VEM There is also a progressive hyperplasia of the capillary bed which reaches strik ing dimensions We believe this lesion is significant and a regular accompaniment of hypertension in the rat (Fig 4)

Metabolic and Humoral Alterations in Hepato-Renal Vasotropic Factors in Renal Hypertension

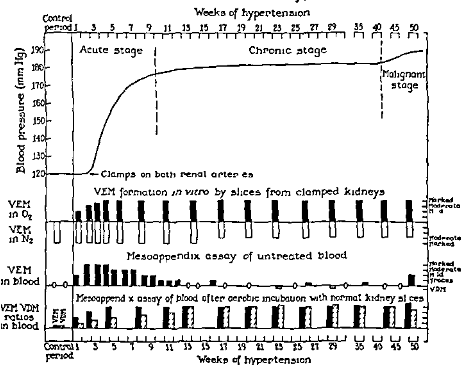


FIGURE 4

The relation of experimental renal to essential hypertension is still controversial It was therefore of interest to look for these same hepa torenal factors in essential hypertension in man utilizing the fractiona tion procedures described above This was done with results which were identical to those obtained in our studies on renal hypertension in the

dog (E Shorr and B W Zweifach, *Tr A Am Physicians 61*, 350 (1948)) VEM and VDM were regularly present in all the cases studied The ratios were generally such as to yield a neutral reaction before fractionation but in a number of instances there was VEM predominance as in the dog This was most likely to occur in the more severe and malignant group Three cases of hypertension due to Cushing s syndrome yielded essentially the same findings These studies were then extended to the hypertension of toxemia of pregnancy, the bloods being fractionated during the peak of hypertension (E Shorr and B W Zweifach, *Program, 22nd Scientific Sessions, American Heart Association, page 7, June 1949*) In all instances large amounts of VEM and VDM were present, sometimes one, sometimes the other factor predominating On the return to normotensive levels following delivery both factors either disappeared or were present in traces

Opportunity was provided in a few instances of essential hypertension to study not only peripheral blood but also blood taken directly by catheters from the renal and hepatic veins The assays showed high concentrations of VEM in renal, and high concentrations of VDM in

TABLE I

Bio Assay of Blood of Patients with Essential Hypertension

Patient Sex Age	Duration Hyper	Blood Pressure	Renal Impairment	Retinopathy	Cereb Phenom	Rat Test of Plasma Untreated	After Kidney Incub
	yrs	mm Hg	0—3+	0—4+		min	min
MK(f) 52	?	172/9[illegible]	0	0	O	Neutral	tr VDM
ET(f) 25	2	180/110	0	0	H	Neutral	VDM 31
RP(f) 26	5	182/110	0	1+	H D	tr VEM	VDM [illegible]
AP(f) 49	11**	250/135	1+	1+	O	Neutral	VDM 29
RC(m) 45	7	172/104	0	2+	H	Neutral	VDM 24
EM(f) 37	3	[illegible]30/130	0	2+	H D	tr VEM	VDM 36
EA(m) 39	6	190/1[illegible]0	1+	2+	O	Neutral	VDM 21
CT(f) 41	1½+	2[illegible]0/135	1+	2+	H D En	Neutral	VDM 33
RP(f) 32	7#	180/106	1+	2+	H	VEM 27	VDM 24
	7##	116/76	1+	2+	O	Neutral	Neutral

** Cushing s Syndrome with malignant adrenal cortical tumor
Aneurysm of L renal artery ## 3 months after L. nephrectomy

TABLE I Vasotropic content in mild to moderate essential hypertension Bio assays carried out on peripheral blood before and after aerobic incubation with normal kidney slices for one hour at 37 5° C to inactivate VEM and unmask VDM H = headache D = dizziness En = encephalopathy

hepatic vein blood the mixed peripheral bloods being either neutral or having some VEM predominance These findings would suggest that at least with respect to these specific defects in the VEM and VDM mechanisms there is no difference between experimental renal and essential hypertension in man (Tables I and II)

TABLE II

Bio Assay of Blood of Patients with Essential Hypertension

Patient Sex Age	Duration Hyper	Blood Pressure	Renal Impairment	Retinopathy	Cereb Phenom	Rat Test of Plasma: Untreated*	Rat Test of Plasma: After Kidney Incub
	yrs	mm Hg	0—3+	0—4+		min	min
HS(f) 48	28***	210/140	0	3+	H S	Neutral	VDM 30
MF(f) 76	22	194/90	1+	3+	O	Neutral	VDM 30
MS(f) 45	8**	260/130	1+	3+	H	Neutral	VDM 26
TE(m) 30	1	180/120	1+	3+	H	tr VEM	VDM 21
MK(f) 41	20**	172/132	1+	3+	H En	tr VEM	VDM 21
43	22***	164/112	1+	3+	O	Neutral	VDM 32
SC(m) 50	4	228/130	2+	3+	O	tr VEM	VDM 19
FH(f) 48	5	240/110	2+	3+	H D En	VEM 21	VDM 27
AG(m) 53	4	238/148	3+	4+	O	VEM 21	VDM 36

* Plasma incubated in 95% O_2-5% CO_2 but without kidney gave same reaction as untreated plasma

** Unilateral sympathectomy *** Bilateral sympathectomy

TABLE II Vasotropic content in moderate to severe essential hypertension Bio assays carried out on peripheral blood before and after aerobic incubation with normal kidney slices for one hour at 37 5° C to inactivate VEM and unmask VDM H = headache S = cerebral accident D = dizziness En = encephalopathy

Fremont Smith What capillary beds have you studied?

Shorr Only the mesentery and omentum

Fremont Smith Do you know whether the hyperplasia is generalized and found in the skin and other parts of the body?

Shorr We do not It is a very striking picture and once we began to recognize it in our animals with experimental hypertension we began to look for it in the large numbers of rats which we use for the mesoappendix tests We found when the mesoappendix was exposed for testing purposes that occasional rats showed the same type of capillary hyperplasia of the vascular bed of the mesentery Several of these animals were saved and subsequently had blood pressure measurements taken on them They were found to have spontaneous hyperten

sion The occurrence of this specific capillary hyperplasia in both spontaneous and experimental renal hypertension is of considerable interest

The importance of the adrenal cortex for hypertension prompted investigation of the relation of the adrenal cortex to the renal VEM mechanisms in dogs, rabbits and rats (B W Zweifach, S Rosenfeld, S Baez and E Shorr, Factors Regulating Blood Pressure, Trans of the First Conference, Josiah Macy, Jr Foundation, New York, p 72, (1947)) It was observed that following ablation of the adrenal cortex the kidney lost the capacity to form VEM either in oxygen or nitrogen This property was not preserved by the maintenance of rats on salt but was maintained by supportive therapy with DCA In addition the kidneys of rats were capped with collodion gauze and the adrenals removed in two stages, the animals being supported by salt (B W Zweifach and E Shorr, *Federation Proc* 8, 175 (1949)) No hypertension developed, there was no hyperplasia of the omental capillary bed and the kidneys were found to have lost their capacity to form VEM However, appreciable amounts of renin were present in the kidneys of these animals When such adrenalectomized rats with capped kidneys were supported by DCA, hypertension developed This interdependence of the renal VEM mechanisms and the adrenal cortex, as well as the very specific metabolic lesions in the renal VEM mechanisms in both essential and experimental renal hypertension, and the specific hyperplasia of the vascular bed of the mesentery in the rat—all these promise to be of great value in elucidating the relationship of the adrenals to hypertension The absolute dependence of hypertension upon the adrenals is clear What remains to be established is whether the adrenals serve as a necessary link in the chain of events leading to hypertension or whether hypertension is primarily the result of adrenal cortical overactivity

Before discussing the relation of these humoral mechanisms to the problems of cirrhosis and edema I should like to comment briefly on the evidence for their participation in congestive heart failure in man In cooperation with Dr Leiter s group at the Montefiore Hospital, we undertook a study of patients with congestive failure due to rheumatic heart disease (R Mokotoff, D J W Escher I S Edelman, J Grossman, L Leiter R E Weston B W Zweifach and E Shorr, *Federation Proc* 8 112 (1949)) In these patients mixed peripheral bloods as well as bloods obtained by catheterization of the renal and hepatic veins were studied for their content of hepatorenal factors Renal function studies including measurements of the oxygen tension of the arterial and venous blood from these organs showed that the mean oxygen tension within these organs was lower than normal and that the A V difference was increased We consistently found VEM in the renal vein blood and

VDM in the hepatic vein blood, the mixed peripheral blood giving either a neutral reaction or a slight predominance of one or the other factor It would appear then that in congestive failure the reduced oxygen tension prevailing in these organs is sufficient to initiate formation of these hepatorenal principles Direct evidence in support of this inference was provided by normal subjects who were breathing a mixture in which the oxygen tension was reduced to 10% After about 10 minutes in this atmosphere, VEM appeared in the renal venous blood The implications of these observations for the hemodynamics of congestive heart failure still await clarification It is well accepted that there is an increased peripheral resistance in congestive failure It is also established that the filtration fraction is increased, most likely due to an increased constriction in the efferent arterioles of the glomerulus It is still a matter of speculation whether the local accumulation of VEM within the kidney may be responsible for this increased efferent arteriolar resistance It was of interest that one case in which VEM was not present in the renal venous blood had no increased filtration fraction However, for the present the role of VEM in vascular behavior within the kidney must remain conjectural

Turning now to the aspects of our studies which are of particular interest to this group I shall describe our findings as to the involvement of these hepatorenal principles in cirrhosis and in edema formation Our initial studies were stimulated by the observation of Dr Paul Gyorgy that animals in nutritional cirrhosis tolerate minor operative procedures, such as liver biopsies, very badly Apparently such animals, as well as human subjects with liver disease, are predisposed to shock Dr Gyorgy kindly made available to us for these studies a group of rats made cirrhotic by a low protein (10%) high fat diet In addition, we also studied a larger group (E Shorr and B W Zweifach *Federation Proc* 7 115 (1948)) Very significant changes were found to have occurred in these animals In contrast with the livers of normal rats their livers usually contained large amounts of VDM VDM was present in the blood just as it is in the blood of shocked animals although the cirrhotic animals were not subjected to any manipulative procedures The mesenteric vessels had a diminished responsiveness to epinephrine such as is seen during the hyporeactive stage of hemorrhagic or traumatic shock when VDM predominates in the blood The liver mechanism for inactivating VDM under aerobic conditions *in vitro* was regularly and significantly impaired It is this defect which is believed to be responsible for the continuous production of VDM by the livers of these cirrhotic rats The kidneys in addition were found to have sustained a very serious derangement of the VEM mechanism as a result of which they were unable to form VEM in nitrogen or formed it in very trivial amounts Thus the hepatic and renal defects relating

to these vasotropic substances were such as to result in a state which predisposed these animals to shock Their inability to call on the renal VEM mechanisms did not permit them to take advantage of the compensatory vascular behavior which this principle induces in response to circulatory stress resulting from shocking procedures The defects in their hepatic VDM mechanism had set up humoral and vascular situations comparable to those seen in animals in the hyporeactive or irreversible stage of experimental shock With respect to VEM and VDM therefore these animals exhibited a condition which could be appropriately termed a hepatorenal syndrome with profound circulatory implications

Best What weight rats?

Shorr In our initial studies we used rats weighing about 160 gms, in the later studies the weight of the rats ranged from 150 180 with an occasional one up to 220 gms

Best Were they not getting any kidney lesions?

Shorr We have taken tissue for sections but they have not as yet been run through They looked grossly normal

Attempts were also made to correct these defects after they had been established by 4 months of this diet Although the number of experiments is not large, two factors were found to correct the renal VEM defect These were methionine supplementation and the administration of DCA Following both procedures the kidneys of these animals regained the ability to form VEM

The relation of diet particularly protein, to the renal VEM mechanisms was reinvestigated in more acute experiments with diets containing 5 and 10% protein as well as fasting (M A Payne and E Shorr, *Federation Proc* 8 125 (1949)) Adequate vitamin supplements were supplied throughout Fasting rats sustained a significant reduction in the capacity to form VEM by the 4th and a total loss by the 6th day of the fast Those receiving the 5% casein diet showed appreciable reductions in VEM formation by the 8th and complete abolition from the 12th through the 39th day With a 10% casein diet VEM formation did not take place after the 8th day DCA failed to prevent this defect whether given prophylactically from the start of the experiments or after the 14th day when this defect was well established It would appear then that the integrity of the renal VEM mechanism is dependent on at least two factors so far studied the dietary protein and the adrenal cortex In acute and extreme protein depletion DCA is unable to restore this defect The implication of these observations for nutritional as well as human cirrhosis are under active investigation As pointed out above the cirrhotic animal by virtue of these defects, is predisposed to circulatory failure This occurs through the loss of the

compensatory effects of VEM and through the presence of defects in the hepatic VDM mechanisms which are comparable to those believed to be responsible for the hyporeactive state of shock which becomes irreversible to transfusion

Two other aspects of cirrhosis may also be involved with these derangements, namely, antidiuresis and edema formation Both have been under active investigation in our laboratory These experiments have served to establish the fact that the administration of partially purified liver VDM concentrates as well as crystalline ferritin or apoferritin with which VDM has been identified, leads to profound antidiuresis in rabbits and dogs receiving oral or intravenous hydration (S Baez, A Mazur and E Shorr, *Federation Proc.* 8, 7 (1949)) The administration of 200 300 gamma of ferritin nitrogen/kg of body weight by intravenous infusion reduces urine flow to the extent of 60 80% during the period of infusion When the oral hydration method is used for producing diuresis there is a significant delay in reaching the peak of diuresis and in excreting the water load These antidiuretic phenomena following ferritin or apoferritin occur with no alteration in systemic blood pressure Animals can be immunized with heterologous ferritin When this occurs, and precipitins for ferritin appear in the blood stream, the antidiuretic effect of ferritin fails to occur (Fig 5)

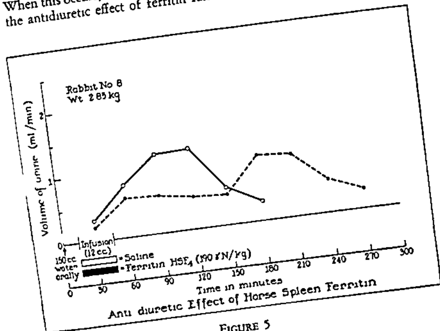


FIGURE 5

Studies are in progress designed to clarify the manner in which this antidiuresis is achieved Three dogs with stalk resection have developed diabetes insipidus These animals which had previously given an anti diuretic response to homologous ferritin failed to do so after the de velopment of diabetes insipidus It is our tentative conclusion that VDM (ferritin) exerts its antidiuretic effect by stimulating the posterior pituitary to discharge its antidiuretic principle

Fremont Smith There was no febrile response?

Shorr There was no febrile response and no change in blood pressure during the experiment

Hanger What is the source of the ferritin you use in the immuniza tion experiments?

Shorr Horse spleen ferritin which is injected into the rabbit We get good precipitin reactions in blood with these heterologous ferritins whereas we do not get antibody formation with homologous ferritins

Best What is the nature of the protein in ferritin?

Shorr There is nothing particularly characteristic of the amino acid composition of ferritin We have done amino acid analyses and have accounted for about 98 percent of the nitrogen without striking any thing peculiar except in one respect As compared with other proteins which are combined with iron the iron in ferritin is tightly bound to the protein The iron also appears to be contained within the structure of the molecule in such a way that it exerts no surface charge Apofer ritin has also been analyzed and there are no significant differences from ferritin in its composition as regards amino acid content

Watson Is it known whether it is similar to the ironbound globulin in the circulation?

Shorr It is quite different The iron in the ironbound globulin of the blood is readily split off whereas the iron is tightly bound within the ferritin molecule and can only be split off by powerful reducing agents

It was now of interest to see whether the findings in human cases with antidiuresis and edema were compatible with the concept that an accumulation of ferritin in the blood as a consequence of hepatic VDM derangements might be responsible for these antidiuretic phenomena in man To this end bio assays were carried out on blood from patients with cirrhosis and antidiuresis and on subjects with nephrosis or in the nephrotic stage of chronic glomerulonephritis (E Shorr B W Zweifach A Mazur and M A Payne unpublished data) In many of these patients edema fluid was also analyzed The bloods and edema fluids were fractionated in the manner previously described for assaying VEM and VDM when they are present in mixtures (Ta[illegible] I & IV)

TABLE III

The Occurrence of VDM in Decompensated Hepatic Cirrhosis

Patient, Sex Age	Diagnosis	Edema	Ascites	Plasma Protein: Albumin	Plasma Protein: Globulin	Plasma Protein: Total	RAT MESOAPPENDIX ASSAY— PLASMA: After Control Incubation with Saline*	RAT MESOAPPENDIX ASSAY— PLASMA: After VDM Inactivation By Incubation With Anti Ferritin Serum**
MC(m) 41	Laennec type	+	+	2 0	2 3	4 3	VDM 18 min VDM 24	neutral
TC(m) 47		+	+	3 0	2 5	5 5	VDM 24 VDM 27	"
JB(f) 39		+	+	3 0	3 4	6 4	VDM 21	
MD(f)		+	+	2 7	3 5	6 2	VDM 18	
MM(f) 55		+	+	[illegible]5	4 2	6 7	VDM 15	
JF(m) 56		+	+	3 2	3 3	6 5	VDM 15	
MMo(f) 48		+	+	4 1	2 7	6 8	VDM 21	
MMu(f) 51		+	+	2 6	3 9	6 5	VDM 21	
MG(f) 51	Cholangiolotic type	0	0	3 8	4 4	8 2	VDM trace	
BS(f) 40	Laennec type (compensated)	0	0	4 7	2 5	7 2	VDM	

* Incubated in 95% O_2-5% CO_2 for 1 hour at 37.5°C

** Prepared by immunizing rabbits with ferritin obtained from human livers.

TABLE IV

The Occurrence of VDM in the Nephrotic Syndrome

Patient, Sex Age	Diagnosis	Edema	Ascites	Plasma Proteins			RAT MESOAPPENDIX ASSAY— PLASMA			EDEMA FLUID	
				Albumin	Globulin	Total	After Control Incubation*	After VEM Inactivation By Incubation With Kidney Slices*	After VDM Inactivation With Anti Ferritin Serum**	After Control Incubation*	After VEM Inactivation By Incubation With Kidney Slices*
BM(m) 20 days	lipoid nephrosis	+	0	1 7	0 8	2 5	VDM 27 min	VDM 28 min	—	—	—
CF(f) 5 yrs	chronic glomerulo nephritis	+	+	1 1	2 5	3 6	VDM 18 min	VDM 15 min	—	VDM trace	—
LF(f) 6 yrs	lipoid nephrosis	+	+	0 8	2 4	3 2	VDM 19 min	VDM 21 min	—	VDM 24 min	VDM 21 min
AM(m) 17 yrs	chronic glomerulo nephritis	+	+	1 7	1 6	3 3	VDM 21 min	VDM 25 min	neutral	VDM 32 min	VDM 29 min
AN(f) 5 yrs	Lipoid nephrosis	+	+	0 4	2 9	3 3	VDM 35 min	VDM 27 min	—	VDM 21 min	VDM 18 min

* Incubated in 95% O_2-5% CO_2 for 1 hour at 37 5°C
** Serum from rabbits immunized with ferritin from human liver

Experimental findings were as follows All subjects with edema and [illegible] VDM in the circula [illegible] VEM was present in the blood or edema fluid In several patients with cirrhosis but without ascites or antidiuresis, VDM was absent or present only in traces Opportunity was provided to study one case of nephrosis at the height of her edema, during treatment with intravenous serum albumin which [illegible]

content of the blood again rose to its original levels These observations are at least compatible with the concept that antidiuresis and edema, in a variety of clinical conditions including cirrhosis, may stem from hepatic defects in VDM mechanisms as a result of which VDM accumulates in the blood and stimulates the posterior pituitary to a discharge of antidiuretic factors There would appear to be parallel derangements of the VEM mechanisms as a result of which they are unable to oppose the antidiuretic action of VDM This latter inference must be regarded as conjectural for the moment and a matter for future investigation

Armstrong In cases of glomerulonephritis which you studied was there any hypertension?

Shorr There was mild hypertension, 140/90 in one case, 160/98 in another However, don t you think that the hypertension in glomerulonephritis is generally ascribed to anatomical changes in the renal blood vessels which increase peripheral resistance?

We are now in a position to summarize the present status of these investigations as to the role of these liver and kidney principles in circulatory homeostasis We have established the conditions under which the metabolism of VEM and VDM is regulated under normal conditions We have found that their metabolism is deranged in a variety of circulatory disturbances such as hemorrhagic and traumatic shock, essential and experimental renal hypertension nutritional and human cirrhosis and in a variety of states associated with antidiuresis The locus of action of these vasotropic principles has been shown to be the terminal vascular bed They exert opposite effects on the behavior of the metarterioles and precapillary sphincters These effects consist in alterations in vasomotion and reactivity of these muscular units of the capillary bed These alterations have a profound influence on the flow through the true capillary bed and on the exchange of fluid which takes

place between the blood within the capillary vessels, and the tissue fluids The preponderance of the vasoexcitor, VEM, makes for capillary ischemia and inward filtration VDM preponderance on the other hand makes for overall capillary flow and outward filtration Unless compensatory vascular adjustments took place in the vascular bed central to the capillaries, VEM predominance would cause tissue dehydration and VDM predominance edema These principles therefore would appear to constitute a new homeostatic mechanism for the regulation of the peripheral circulation They would appear to constitute a major element in the homeostatic forces whose end is the preservation of the stability of the internal environment In a variety of circulatory disturbances, these vasotropic systems undergo specific derangements of such character as to suggest that they are related to a number of phenomena characteristic of these circulatory states Several environmental factors have been shown to lead to derangements in these hepatorenal mechanisms Those already studied are reduced oxygen tension, low protein intake and adrenal cortical hormones Low protein diets impair the integrity of both the renal and hepatic vasotropic systems The adrenal cortex is necessary for the integrity of the renal VEM mechanism [illegible] th hepatic and renal systems [illegible] be concerned not only with [illegible] also with the renal excretory [illegible] established in animals and [illegible] appear to be mediated through the posterior pituitary The possibility has been advanced that oliguric phenomena in human disease may be due to derangements in the VDM and VEM mechanisms as a result [illegible]

It is appreciated that much remains to be done before a causal relationship can be established These principles act in a chronic and subtle manner quite different from other vasoactive substances such as renin and angiotonin whose effects on blood pressure are more acute

Markowitz Do you know Trueta s work and what do you think about it?

Shorr I have re[illegible] graph of [illegible] his associates and spent a whole even[illegible] os and cons of the Trueta mechanism [illegible] th the [illegible] ssion The pros seem to have de[illegible] this postulated mechanism The co[illegible] relev[illegible] l mechanism, [illegible] problem of [illegible] in [illegible] evidence [illegible] it was the [illegible] extraction [illegible] extreme [illegible] is and

man Thus we have an anatomical pattern for which no functional corollary has as yet been demonstrated at least to the satisfaction of most of the workers in the field of renal physiology

Markowitz He gave evidence in his book which he published with Barclay, Franklin and Pritchard It seemed a very convincing story I spent a day at Oxford watching the work going on and it was very impressive

Shorr I think the present difficulty is determining its role in renal function Even in severe experimental shock, with reductions in blood flow down to 3% of the normal, Van Slyke showed no change whatsoever in the PAH extraction ratio

Hanger Could I ask you your opinion as to the mechanism of ascites in cirrhosis? Do you think it is due to an alteration of the capillaries of the peritoneum which disturbs the reabsorption of fluid, or do you think it is all an antidiuretic effect?

Shorr I am inclined to favor the participation of positive antidiuretic factors as basic to the water retention with the localization of fluid within the peritoneal cavity as a result of local factors such as intra hepatic resistance to portal blood flow But this latter effect we would regard as subordinate to the factors influencing the renal excretion of fluid and salt There is also the possibility that the decompensatory effects of the chronic predominance of VDM on the capillary blood flow would by favoring the hydrostatic pressure over the osmotic also contribute to the formation of tissue edema.

Hanger That is what I wanted to know

Shorr There is also some evidence which we hope to supplement by more experiments that the hypervolemia of cirrhosis and infectious hepatitis may be related to VDM predominance in blood Through the

Zweifach and E Shorr unpublished data) One is tempted to interpret this situation as follows for some unknown reason the granulosa tumor affects the hepatic VDM mechanism and leads to VDM formation As the VDM accumulates in the blood stream it progressively opens up the capillary bed In order to fill the capillary bed and prevent circulatory collapse, there is a parallel increase in the blood volume This very interesting aspect of our studies needs amplification and we hope to report to you more fully at subsequent meetings of this group

Fremont Smith Have you any evidence that VDM could still have an antidiuretic effect, if it were masked by equivalent amounts of VEM?

Shorr We suspect that in the presence of equivalent amounts of

VEM VDM would have no antidiuretic effect This is at present a matter of inference in that our patients with both VEM and VDM in blood show no delay *in excreting the water load* When we have obtained VEM in sufficiently pure form we shall carry out direct experiments bearing on this point

Fremont Smith Did you have one case of mild hypertension in the *nephrotic group? This one case with a blood* pressure 140/90 did not seem to fit in that it had antidiuresis with both VEM and VDM in the blood

Shorr There were 2 patients with glomerulonephritis and mild hyper*tension But there was no VEM* present in either patient s blood only VDM

Fremont Smith Is VEM not present in the hypertension of glomerulonephritis?

Shorr We cannot say very much about glomerulonephritis We have studied a few severe cases in uremia but their bloods were so toxic for our animals that we could not assay them This same toxic effect is also exerted by blood from animals in the terminal stages of malignant renal hypertension

Fremont Smith What about eclampsia? Is that the same?

Shorr In our eclamptic cases whenever the bloods proved toxic we were able to assay them after dilution with saline

Fremont Smith With what results?

Shorr Both VEM and VDM were regularly present sometimes one sometimes the other predominating at the peak of the hypertension before delivery As I described above with the fall in blood pressure after delivery these factors disappeared or were present in only trace amounts

Hoffbauer How potent is ferritin as an antidiuretic? Might this test be used in the place of the rat mesoappendix assay for VDM?

Shorr The rat test will detect ferritin in amounts of 0 0005 micrograms of nitrogen To obtain an antidiuretic effect we must inject 200 300 micrograms/kg Hence the antidiuretic test could not possibly be used for the assay of VDM in blood because the amounts present in the body fluids are more on the order of 0 001 micrograms/cc

Watson Is ferritin plentiful enough so that you might propose to try it out on the human subject?

Shorr We are accumulating a stock pile of crystalline human ferritin for just such purposes

Gyorgy You probably could not use the antiserum for treatment of human disease because the supply might be quite limited

Shorr We hope that with the help of our friends we will eventually

accumulate enough ferritin to produce enough antiferritin serum for human use We have already begun experiments with antiferritin serum in animals but it is too early to speak about that

Watson There is no evidence of ferritin or apoferritin in urine is there?

Shorr Dr Mazur has accumulated some evidence that small amounts of ferritin, of the order of 10 to 15 micrograms of ferritin N per 24 hours may be excreted by the human subject This amount of ferritin is much too small to account for the antidiuretic effects obtained with urine concentrates in cirrhosis by Ralli et al (E P Ralli, J S Robson, D Clarke, and C L Hoagland *J Clin Investigation 24*, 316 (1945)) and other workers

Fremont Smith You don t think that ferritin is antidiuretic by virtue of its action on the kidney itself but through the release of antidiuretic factors from the posterior pituitary

Shorr That is our present view I can t say as yet that it has no direct action on the kidney but our results so far suggest that its oliguric effects are mediated through the anterior pituitary

Markowitz Do these dogs have polyuria?

Shorr Yes

Markowitz What was done?

Shorr A resection of the stalk

Markowitz You could still get diuresis

Shorr Four animals were operated upon One by Dr Kendrick Hare and three by Dr Silvio Baez of our laboratory All four had the polyuria which is usually described to follow this procedure

Markowitz How much diuresis did you get?

Shorr On the third or fourth day after operation, diuresis was very marked—up to 2 3 liters per 24 hours The polyuria was gradually reduced and after a month or so was about 1 liter per 24 hours This is from 2 to 3 times the usual urinary output of the dog

Markowitz I have not been able to do it I ll have to try again

Shorr We were greatly helped by the advice of Dr Kendrick Hare who has been carrying out these procedures for many years

Markowitz I have often wondered why a hypophysectomized dog did not have diabetes insipidus

Shorr Diabetes insipidus does not follow total hypophysectomy You have to leave in the anterior lobe

Markowitz That explains the whole thing I am sorry I was not clear about that in my mind

SECTION V

THE METABOLIC BEHAVIOR OF THE EVISCERATE RAT

Ingle Gentlemen, my interest in the eviscerate rat is concerned with two general problems First, what metabolic adjustments can this preparation carry out? Second, what factors influence the survival of the eviscerate animal? I think that this latter problem is one of the most challenging in physiology

We have adequate replacement therapy for vital organs such as the pancreas, the adrenal cortex and the anterior pituitary, whereas the heart, lungs, and brain are essential for obvious reasons The liverless animal does not survive for very long and the causes of death have not been determined Could we prolong the survival of the eviscerate rat by improved intravenous feeding and would it be possible to extract anything from liver itself which would prolong survival?

I started this work at the Mayo Institute twelve years ago You may well guess that my interest in liver was stimulated by Drs Mann and Bollman My beginning studies involved certain difficulties In order to keep the animals living I had to inject them frequently with glucose and in order to determine survival I had to either spend the night in the laboratory or carry the animals back and forth between the laboratory and my home My friends in the laboratory exhibited unsympathetic amusement and the members of my family were neither sympathetic nor amused I dreamed of a time when I could have instrumentation which would permit us to feed the animals by continuous intravenous injection and to record the time of death through the automatic measurement of some physical change

If I may have the first slide, we will skip a number of years up to the time when I acquired such a device (Figure 1) These are six eviscerate rats enclosed in a box with temperature constant at 26 5±0 5 Centigrade The animals are fed intravenously by constant infusion We determine the time of death by amplifying the D C potential

activates a relay switch which in turn

ie Micromax recorder The Micromax

six rats We can record accurately the

time that the heart stops beating within six minutes

Now for a little more detail about this procedure We anesthetize the animals with cyclopal, which is a rapidly acting barbiturate Intact rats recover rapidly from this anesthetic but the liverless rat does not recover from any kind of barbituric anesthesia, although it does recover

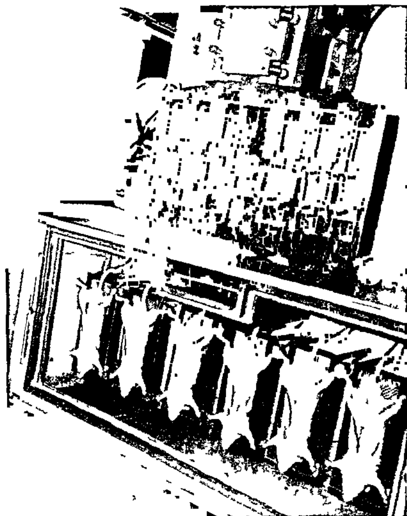


FIGURE 1

from ether The animals are placed on their backs upon a little table above a funnel and a collection flask so that we can recover the urine The continuous injection machine was designed and built in our shops It handles six syringes and its blocks will accommodate any size syringe from a 1cc to a 50cc capacity Syringes which will deliver their volume with the standard stroke of 65 mm must be ordered to a specified

diameter The machine has several different speeds so that the volume which can be delivered per unit time can be varied over a wide range In all of the experiments to be described we have used a standard rate of delivery, 20cc per 24 hours The solutions all contain 0 9 percent sodium chloride with and without insulin and with various glucose loads Body weight was constant at 250 grams

(Slide Table I) We have recently completed a study of the blood changes in these animals after 48 hours of infusion, a time at which they are approaching death The glucose load was 44 mgms of glucose per 100 grams of rat per hour (44/100/h) and the insulin dose was 4 units of regular insulin (Lilly) per 24 hours per rat I have listed here some of the most striking changes that we have seen There are other changes which may be important although less striking The prothrombin time is short at the beginning of the experiment but after 48 hours the blood will not clot at all At the time of death there are many diffuse hemorrhages which almost always involve the bladder, frequently the kidneys and occasionally the lungs I have even seen hemorrhages in the brain The white cell count is markedly reduced *This may be related to the fact that these animals are non resistant to* infection Dr Armstrong mentioned yesterday that the rat is highly resistant to infection This is true of the normal rat but not of the liver less rat Within 24 hours the blood the body cavities and even the bone marrow are heavily charged with bacteria and we have not yet been fully successful in our attempts to preserve asepsis in our opera tions We can reduce the bacterial growth per unit time in all our animals and can carry some of them through the experiment without any bacterial infection The extent of infection is certainly a factor which influences the time of survival

TABLE I

Some changes in the blood of eviscerate rats

	Initial	48 Hours	
Prothrombin Time	15	Infinite	(Seconds)
White Cell Count	11 6	2 8	(Thousands/CMM)
Total Plasma Protein	5 8	3 3	(GMS/100/CC)
Bilirubin	0 0	4 4	*(MGMS/100/CC)*
CO_2 Combining Power	62 5	35 0	(Volumes/100/CC)

Shorr Have you ever used penicillin?

Ingle No *After the experience of Dr Markowitz* we will certainly try that The plasma protein is reduced This may be related to the development of ascites in the eviscerate rat Some of them virtually drown in their own fluid since the chest cavity fills so completely that

respiration may be impossible If the fluid is drained from the chest the animal may survive for a few hours longer but will eventually die for other reasons We have infused the rats with colloids (gelatin) in an effort to keep up the osmotic activity of the blood but it has not affected survival Gelatin seems to escape rapidly from the blood

There is no measurable amount of bilirubin in normal rat blood but following evisceration the animal becomes severely jaundiced There is a definite tendency to develop acidosis following evisceration

Hanger Did you study blood platelets? To my knowledge no one ever studied blood platelets in eviscerated animals Do your smears give you any indication of a change?

Ingle Miss Prestrud has carried out the hematological studies of our eviscerate rats Although platelet counts have not been done, it is her impression that the number of platelets were greatly reduced following evisceration Until a more precise study has been carried out it will not be possible to reach a conclusion regarding platelets

Armstrong Can you indicate on this slide where the plasma protein disappears? That seems to be faster than one would expect it from the isotope studies of its disintegration

Ingle The fluid which accumulates in the body cavities contains significant amounts of protein It seems probable that it has come from the blood

The other day at Detroit, Dr Roberts discussed changes in plasma proteins in the eviscerate rat and said that they did not find plasma proteins in the fluid which they recovered from the body cavities but their experiments were terminated within 24 hours as compared to 48 hours in our studies Dr Roberts noted that the albumin of the blood

the presence of fibrin did show that there was a more rapid loss of albumin than of globulin in our eviscerate rats and that our data are in agreement with the data of Roberts although the experimental conditions were rather different Our animals were infused with glucose and insulin and the animals of Roberts were not

Fremont Smith How much weight do the animals gain?

Ingle About 10 grams on the average

Kinsely Perhaps everyone else knows, but would you mind describing the surgical operation done on the animal in evisceration, how you do it, what do you take out?

Ingle We remove the liver and the entire gut leaving only the kidneys and the adrenals and in some experiments we remove them also It is

a two stage operation The first stage involves total ligation of the vena cava between the liver and kidneys so that the animal builds up a collateral circulation The second stage or actual evisceration is carried out two or three weeks later

To come back to the question of the missing plasma protein, Dr Roberts thinks that it may be used by the kidney for renal gluconeogene sis This must be considered

Shorr Do you know whether the lactic acid content of the blood or tissues rises?

Ingle We have not determined that recently I think that Dr Bollman remembers the few experiments that we did when I was with him a number of years ago As I remember, there was no increase in the lactic acid of blood and muscle within the first few hours As a matter of fact, the increase in lactic acid which follows the stimulation of muscle is not sustained The animal has the ability to utilize lactic acid in the absence of the liver Would you agree to that Dr Bollman?

Bollman That is essentially what we have found in the eviscerated as well as in the hepatectomized rat and in the eviscerated dog Im mediately after operation there is a rise in the lactate content of the blood and also of the muscle This rise is related to the anesthesia and surgery as it is similar to that found in sham operated animals On recovery from the operation the lactate levels of blood and muscle return to normal in the sham operated animals but in the eviscerate or liverless animals the level may decrease but does not return to normal levels There is usually a terminal rise of lactate which is of larger magnitude With exercise or muscle stimulation there is the expected increase in the lactate content of the muscle and blood and with rest or continuing muscular activity there is a disappearance of the lactate as occurs in the normal animal The level in the muscle and blood of the eviscerate animal returns to its pre exercise level which is higher than that of the intact animal Large amount of injected lactate will also disappear from the blood of the eviscerate animal There seems to be no real impairment of the animal s ability to metabolize lactate

Shorr I was thinking in terms of the long experiment in which the circulatory phenomenon might be the limiting factor rather than the ability to oxidize in the presence or store in the presence of insulin and glucose If you have a limiting factor in terms of the oxygen supply to the tissues in the course of the deterioration of the animal you might get a lactic acid again I know Soskin, for example, in evisceration experiments, got back lactic acid The values rose at a very extraordinary rate, if you remember

Bollman You are quite right As the animal fails in the shock like conditions with low blood pressure and blood volume there is a marked

rise in the lactic acid content of the blood and muscles This has little to do with the liver but is more related to the relative anaerobiosis of the tissues Of course if the blood could be circulated through a normal liver most of the lactate would be removed but the impairment of circulation and not the absence of the liver is the cause of the increased lactic acid

Fremont Smith Do you know what happens to the blood volume? Does the blood volume stay about the same?

Ingle We do not have any satisfactory measurements of blood volume The hematocrit does not change and the red cell count does not change but it is possible that there is a significant shift in the volume of circulating blood

Fremont Smith What would you say is the blood volume of these animals normally or after you eviscerated them? Would it be 15 16 cubic centimeters?

Ingle We are able to recover 6 to 8 cc from these animals at the end of 24 hours which is not as good a recovery as you can get in a normal animal

Goldblatt What weight?

Ingle 250 to 260 grams

Shorr That would be 15 to 16 cubic centimeters

Fremont Smith What I was trying to get at is that the animal gained 7 grams in weight or 7 cc of water and with the blood volume which you anticipate it seems to me that the gain in weight is enough to dilute your plasma by this much assuming that you leak whole plasma out into the pleural cavity and other tissues I think one could probably make at least a guess that if there is a gross permeability change and leakage of whole plasma and if the hematocrit does not change (*i e* blood volume remains about the same after the operation) then the retention of fluid in the animal as measured by the gain of weight of 7 grams would be enough to account for the dilution of the plasma proteins

Ingle Yes That is a reasonable possibility

Shorr How much have you lost in the body cavities?

Ingle We have not measured that

Shorr Can that be 5 or 6 cubic centimeters?

Ingle It might be

Shorr So that might reduce the blood volume

Fremont Smith But they have gained 7 cc of water which would be enough to restore the blood volume to its original level But whole

degrees the survival time is decreased below the optimal values obtained at 26 5 degrees

Shorr That happens to be the temperature that Dr Simms, who is doing long time aging studies on rats, has chosen as optimal for the maintenance of the rat, 78 degrees

Ingle (Slide, Table III) Temperature has a marked effect upon the glucose requirement of the eviscerate rat At 26 degrees C the glucose requirement over a 3 hour period following evisceration is 64 mgms of glucose per 100 grams of rat per hour (64/100/h), but increasing the temperature to 38 degrees C brings the glucose requirement up to 150/100/h We have explored this temperature range thoroughly and a change of even 1 degree C will make a measurable difference in the glucose load which the animal can tolerate

TABLE III

The effect of temperature upon the tolerance of the eviscerate rat for glucose

Degrees C	Glucose Requirement for a 3 hour period mgms/100/h
26	64
38	150

Gyorgy How is this glucose measured?

Ingle We determine the glucose load which sustains the blood glucose at its initial level of approximately 100 mgms percent We had not worked at this problem very long before I began to bring in one of my other major interests, that of the adrenal cortical hormones Our thinking about the nature of adrenal hormone action has become rather rigidly channelized and the textbook story would explain adrenal cortex physiology in terms of salt and water balance and in terms of hepatic gluconeogenesis from protein The story of the relationship of the adrenal cortex to organic metabolism is something like this there is an increase in the need for the cortical hormones during any type of stress and the gland responds with an increase in its secretory activity The increased output of the hormones stimulates gluconeogenesis from protein in the liver thereby providing the extra carbohydrate energy needed to meet the increased requirement for carbohydrate energy during stress The liver is considered to be the site of action of the cortical hormones I doubt that any of the active investigators in the adrenal field would subscribe to such a simple story which does, never theless, appear in textbooks and in the classroom We set about to

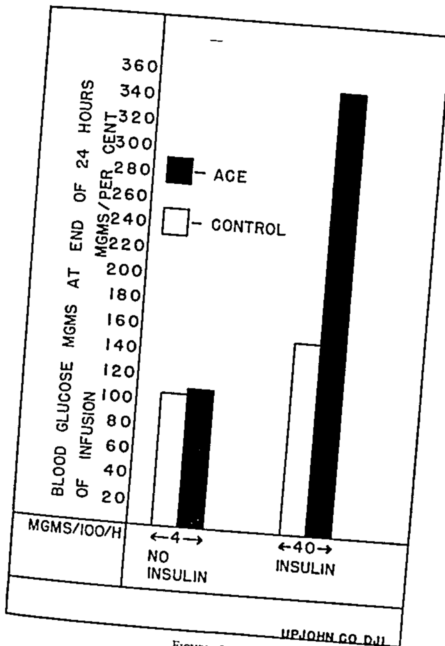

BLOOD GLUCOSE MGMS AT END OF 24 HOURS OF INFUSION
MGMS/PER CENT
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
– ACE
– CONTROL
MGMS/100/H
←4→
NO INSULIN
←40→
INSULIN
UPJOHN CO DJL

FIGURE 2

determine whether adre...
in the absence of the l ... showing
that the 11 17 oxygena... pounds of the adrenal cortex are dia betogenic even in normal rats and can cause the pouring out of much larger amounts of glucose than could possibly be accounted for in terms of gluconeogenesis from protein It seemed necessary from our data and from that of other investigators to conclude that the cortical hor mones must affect some phase of carbohydrate utilization I ...
the term utilization in a broad ...

... Figure 2) We studied the effect of adrenal cortex extract upon the glucose tolerance of the eviscerate rat over a 24 hour period The glucose requirement of the rat falls during the first several hours following evisceration so that a glucose load of 4/100/h is sufficient to sustain a normal level of blood glucose by 24 hours When ... lin is added the glucose requirement ... can
... the
... not affect glucose tolerance
... absence of insulin but in the presence of insulin it caused marked hyperglycemia and glycosuria in most of the animals

(Slide Figure 3) We anticipated that the effect of adrenalectomy in the eviscerate rat should be more striking in animals treated with insulin than without insulin but the opposite was true In eviscerate rats given a glucose load of 4/100/h the removal of the adrenal glands increased the rate at which they rem...

... animals survived but the ... for blood glucose were below normal whereas the non adrenalec tomized eviscerate rats developed hyperglycemia Adrenalectomy in eviscerate rats given a glucose load of 40/100/h with insulin failed to cause hypoglycemia in fact there was some decrease in glucose tolerance The changes were not great but we have seen it occur re peatedly and I have no doubt that it is a true difference Whenever you explore the dimension of load response in adrenalectomized animals when the load approaches a maximum the adrenally insufficient animal fails first In this experiment we pushed the glucose tolerance to its peak with insulin and the adrenally insufficient animal could not quite attain this peak At least that is the most rational general explanation that has occurred to us up to the present

(Slide Figure 4) We at first assumed that the effect of adrenalectomy upon the glucose tolerance of the eviscerate rat was due entirely to the removal of the adrenal cortex We now have data which provide a

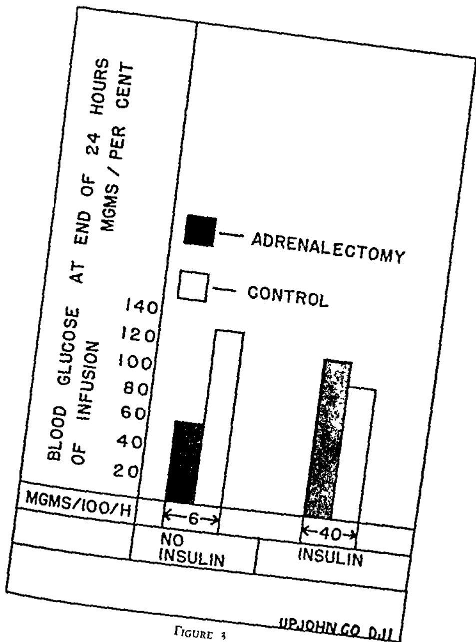

BLOOD GLUCOSE AT END OF 24 HOURS
OF INFUSION MGMS / PER CENT
ADRENALECTOMY
CONTROL
140
120
100
80
60
40
20
MGMS/100/H
6
40
NO INSULIN
INSULIN
UPJOHN CO DJI

Figure 3

basis for suggesting that the absence of the medulla may have been a factor I was taught, and most of the textbooks teach, that epinephrine does not affect the level of blood glucose *in the absence* of the liver and yet there was quite a debate in the literature over this point by C F Cori, (Physiol Rev *11*, 143 (1931)) who claimed an *extra* hepatic action of epinephrine upon blood glucose and by S Soskin (Amer J Physiol *81*, 382 (1927)) who denied it H P Himsworth and D B McN Scott (J Physiol *93*, 159 (1938)) reported that *epinephrine* accelerated the rate at which glucose disappeared from the blood of animals in which the liver was excluded from the circulation We have results that will agree with all three conclusions, depending upon the experimental conditions When the test period was limited to 2 hours, the administration of epinephrine with glucose and without insulin in the infusion fluid did not affect the level of blood glucose significantly When epinephrine was given in the presence of insulin it caused a striking rise in blood glucose Then as we repeated these

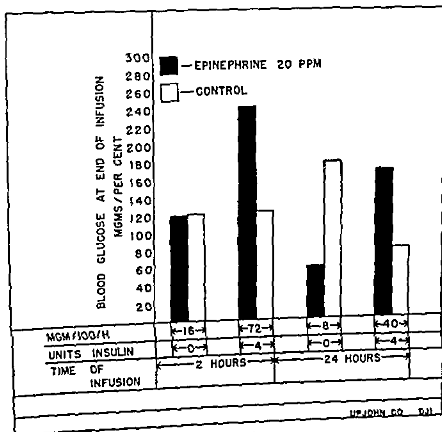


FIGURE 4

experiments over a period of 24 hours we found that epinephrine given in the absence of insulin did indeed accelerate the rate of disappearance of glucose from the blood but in the presence of insulin the administration of epinephrine caused a rise in blood glucose

Best Could that be related to blood pressure?

Ingle We have not measured the blood pressure in these experiments but as I shall mention in a moment we have used a derivative of epinephrine which has a vasodepressor action and which gives the same story

Shorr What happened to the carbohydrate content of muscle under these experimental conditions? Do you think that the epinephrine effect on the blood sugar might be due to the muscle having a high content of glycogen which could give rise to lactic acid and that this did not take place in the presence of insulin?

Ingle We have not measured the tissue glycogen at all, Dr Shorr That is a possibility In describing these changes in carbohydrate tolerance we cannot make any claim to know what happened to the carbohydrate that we administered or what shifts in carbohydrate stores occurred

(Slide Figure 5) This gets more complicated as we go along There are many substances that affect the glucose tolerance of the eviscerate rat In fact we have not yet studied anything that fails to do so We have used posterior pituitary extract in these experiments and obtained exactly the same story that we obtained with epinephrine The results are not quite as striking but are all consistent The administration of posterior pituitary extract to rats given glucose and insulin for 2 hours causes a decrease in glucose tolerance Without insulin there was little or no effect Over a period of 24 hours the administration of posterior pituitary extract caused some decrease in glucose tolerance in the presence of insulin and some increase in tolerance in similar animals not given insulin We repeated the experiments using Pitressin and obtained exactly the same story Preparations of Pitocin gave small effects but it was found that this preparation of the oxytocic principle contained about 20 percent as much pressor activity as did the Pitressin Up to this point it seemed probable that the effect of these hormones upon glucose tolerance was closely related to pressor activity However we carried out an additional experiment using Isuprel which has a depressor action and the response to this pharmacologic agent was exactly the same as was obtained with epinephrine We still suspect that our results on glucose tolerance are based upon some action of these drugs upon the circulatory system

The point which I wish to make from these studies is that the eviscerate animal is capable of responding to these pharmacologic agents

Whether these results deserve any physiological interpretation I don't know, but the eviscerate animal is capable of adjusting to these hor mones, peculiar as the adjustments may be, and the kind of effect that we get is dependent upon the time over which we observe the animal and whether or not insulin is present

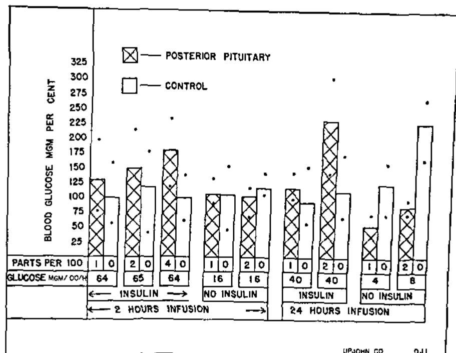


FIGURE 5

Bollman What are the dots on the slide?

Ingle They show the range, the highest and the lowest values There is quite a lot of variability in most of them Here we happen to come out with a very small range, much smaller than is found at 24 hours We were just lucky in that series There are 20 pairs of animals repre sented in each group

(Slide, Figure 6) It has been known for many years that blood amino acids increase after removal of the liver It was demonstrated a few years ago by E G Frame and J A Russell (Endocrinology, 39, 420 (1946)) that the administration of insulin suppresses the rise to a significant extent In these experiments we were able to suppress the rise in blood amino acids completely by the administration of insulin The initial value for whole blood is approximately 10 mgms per cent, and at the end of 24 hours without insulin the average value is nearly 30, but when insulin is given it is held at the initial level The extent

of the rise and the degree to which insulin inhibits the rise is relate[d] to temperature as J A Russell and M Cappiello (Endocrinology, 44 127 (1949)) have recently demonstrated

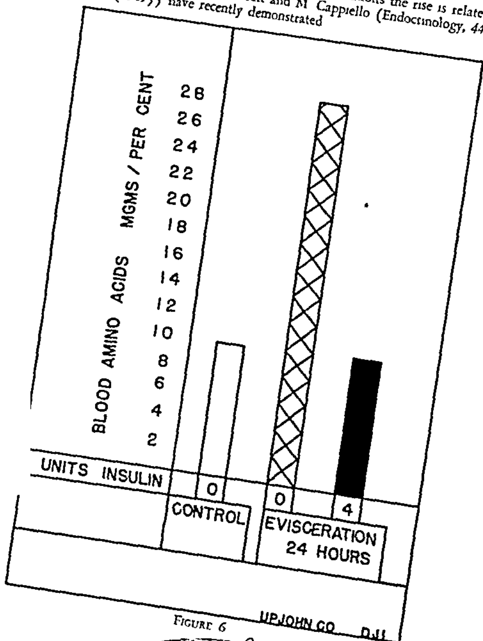


Figure 6

(Slide Figure 7) We now come to a technic which I began to develop at the University of Minnesota in 1931 and which has now come through quite a period of evolution It is a method for measuring the ability of the rat to sustain work output in response to faradic stimulation The rat is anesthetized with sodium phenobarbital the gastrocnemius muscle is weighted with 100 grams and is stimulated to contract 5 times per second The total distance that the weight is lifted is recorded on the work adder We are now interested in making the rat do just as much work as possible and we are passing the stimulus through both back legs thereby activating all of the muscles of the legs and back Even this did not satisfy us We have stimulated through *the entire body of the animal and through various parts of the body* but such animals fatigue rapidly and die within an hour The activation of both back legs causes the animal to develop hypoglycemia and death will occur from hypoglycemic shock in some animals unless glucose is administered Our present setup permits us to work 12 rats simultaneously and to feed them by constant intravenous infusion

Shorr For how long a period of stimulation each time? Is this continuous?

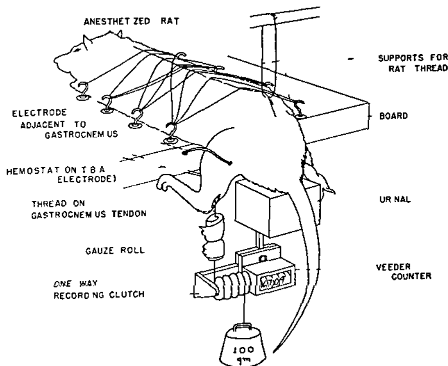


FIGURE 7

Ingle No, intermittent, five times per second The duration of each stimulus is about 20 milliseconds

Shorr This is going on continually throughout the study?

Ingle Yes

(Slide, Figure 8) These data show that adrenal cortex extract can affect the work performance of the rat in the absence of the gut, liver, kidneys and the adrenal glands Following evisceration all the animals were given enough glucose to prevent any degree of hypoglycemia, yet there was a striking effect of adrenal cortex extract upon the ability of these rats to work These data add to our evidence that the action of the adrenal cortical hormones, particularly upon vigor is not mediated by the liver or by any of the intra abdominal organs At the present time I do not have very much of an idea about the nature of the processes which the cortical hormones primarily affect Actually if you should go through the literature on adrenal cortex physiology seeking some meta bolic process which remains normal under all conditions of adrenal hormone insufficiency or excess the search might be in vain

Armstrong Does this require whole adrenal extract or do any of the fractions such as Doca affect work capacity?

Ingle We have used only whole adrenal extract in the eviscerate rat, but we know from studies on adrenalectomized rats that the 11 oxygenated and especially the 11 17-oxygenated adrenal compounds are far more potent than 11 desoxycorticosterone in sustaining work output I might say that the work test was the procedure used to first demon strate to Dr Kendall s group that they were in possession of an active adrenal steroid Dr Mason isolated 17 hydroxy 11 dehydrocorticosterone in that laboratory in 1935 We demonstrated its activity immediately in the work test but nearly two years passed before any other laboratory found the compound to be active When 11 desoxycorticosterone became available, this was the first test used to demonstrate that its biological properties differed from those of the 11-oxygenated compounds It is of considerable personal interest to me to note that 17 hydroxy 11 dehydro corticosterone (Mason s Compound E) is the most sought after of the corticosteroids and that its use is now related to some of the most exciting developments in the clinic as well as in the laboratory

Best When you said that these changes were not related to the supply of carbohydrate you did not eliminate the effect of sugar

Ingle That is true Certainly the exhaustion of carbohydrate stores in these adrenally insufficient animals can be a limiting factor When it is corrected the animal will still fail for other reasons It can work a little longer when it is given glucose than when it develops hypo glycemia but when glucose is given other deficiencies which we do not understand become manifest

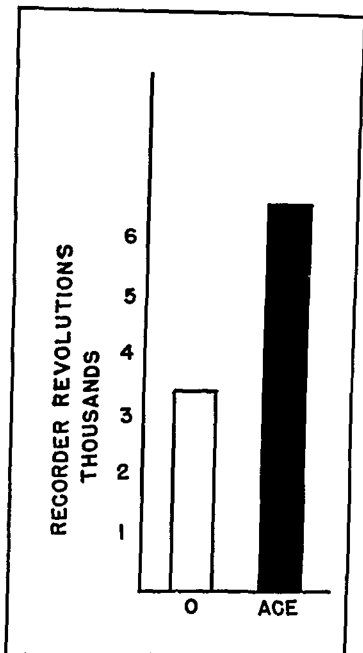


FIGURE 8

(Slide, Table IV) This final slide brings us to a story which seems *to me to* be the *most interesting that we have found* We have studied the effect of muscle work upon the tolerance of the eviscerate rat for

glucose during a period of 2 hours In resting animals without insulin the glucose tolerance is best represented by a load of 16/100/h If we add insulin, the glucose requirement is increased to 64/100/h If we stimulate one leg without adding insulin the glucose requirement is increased from 16 to 48/100/h and if insulin is added the requirement goes to 110/100/h Now if we stimulate all of the musculature of both back legs without adding insulin the glucose requirement is increased ten fold over the resting state When insulin is added, there is another significant increase in glucose tolerance This may be entirely fortuitous but it is nevertheless interesting that the increment which insulin has made in the glucose tolerance in each of the three experimental conditions is just about the same in each case

TABLE IV

The effect of muscle work upon the tolerance of the eviscerate rat for glucose during a period of two hours

Muscle Stimulation	Insulin Units Per 24 Hours	Glucose Tolerance mgms/100/h
0	0	16
0	4	64
1 Hind Leg	0	48
1 Hind Leg	4	110
2 Hind Legs	0	160
2 Hind Legs	4	220

Markowitz I think the point is important How long has that preparation been without any insulin when you began that experiment?

Ingle The pancreas was removed by evisceration and immediately following operation the animal was subjected to the stimulation of muscle for two hours

Markowitz It is not diabetic muscle?

Ingle No but I will try to answer that question in a few moments The working rat without insulin tolerates 160/100/h of glucose and the work output is normal When insulin is added hypoglycemia ensues and the rate of work is sharply decreased These observations suggest but do not prove that the pathways of glucose utilization favored by insulin are competing with the contracting muscle for the carbohydrate We do not know what is happening to the glucose but its disappearance must be related to the activity of the muscle This suggests that the working muscle can utilize glucose perhaps in an optimal manner in the insulin-deficient rat

This brings us to the question just raised by Dr Markowitz What is the story on diabetic muscle? We have used rats having alloxan diabetes and have selected animals which excrete as much glucose as is available from the carbohydrate glycerol of fat and protein (estimating

would show up any kind of physiological deficiency any departure from normal and now we have sometimes thought that these totally diabetic rats work a little better than non diabetic rats The most work that we have obtained per unit time has been from diabetic rats In respect to glucose tolerance the effect of work upon glucose requirement is essentially the same as we found in the above experiment All of the values are shifted down a little The resting diabetic rat without insulin requires about 12 mgms of glucose per 100 grams of rat per hour instead of 16/100/h work causes a marked increase in the rate of disappearance of glucose from the blood not quite as great as in the previous experiment but the change is still very striking

This brings me to the end of the story Perhaps I should mention a little of what we hope to do Our studies as you have seen are exceedingly superficial We do not know what is happening to the carbohydrate except that it disappears from the blood I am very hopeful that Dr Stetten is going to come in with us on certain of our experiments that have to do with the metabolic behavior of the eviscerate animal Certainly he is prepared to give these studies the depth that they deserve For our studies on the survival of the eviscerate rat we now have apparatus that will accommodate 12 animals at a time and will permit us to push ahead more rapidly to determine the effects of other food factors in the infusion fluid and to test the possibility that the liver contains or secretes something that may affect the survival of the liverless animal

Fremont Smith There are 500 chemical functions of the liver

Ingle With that thought in mind it might be appropriate for me to close these remarks with a few lines from the Walrus and the Carpenter I have never quite become adapted to my inability to probe into each one of the myriads of problems that are presented in research

The Walrus and the Carpenter
Were walking close at hand
They wept like anything to see
Such quantities of sand
If this were only cleared away
They said it would be grand!

If seven maids with seven mops
Swept it for half a year,
Do you suppose, the Walrus said
That they could get it clear?
I doubt it, said the Carpenter,
And shed a bitter tear

DISCUSSION

Stetten I would like to ask a question in regard to the efficiency of muscle work It would seem to me you have all the data here whereby you can compute the efficiency of the muscle work in the hind leg of the rat under various circumstances In other words did I understand it, with your Veeder counter technic plus the information about the weight you could calculate work directly if the drum was calibrated In one or two legs it came out pretty nearly 100 mg of glucose per hour per leg I wonder if you have some computations as to how much of that glucose oxidized to completion was converted into mechanical work

Ingle We have not Dr Stadie once made some calculations which did not come out according to his expectations and which may have been due to an error inherent in our recording device We have recently had our physics department determine the extent of error There is a substantial amount of coasting in the recorders We are able to keep the maximum relative error between recorders down to 15 percent and the average error is very much smaller than that well below 10 percent

Shorr Could I ask you to dilate a bit on the diabetic animals? How was the diabetes achieved? Were these completely eviscerated? Were the kidneys in or out? Was there loss of carbohydrate in the urine?

Ingle These animals were alloxan diabetic rats We have used some that were made diabetic by pancreatectomy During the two hours of work the animals have not excreted glucose in the urine

Markowitz What evidence have you that the alloxan diabetes was a nearly total diabetes or total diabetes?

Ingle We followed the glucose excretion of these rats that are force fed Our diabetic rats are force fed a fluid diet by stomach tube twice each day so we have exact control over the food intake We know its carbohydrate content its protein content and our estimates of the amount of glucose that these animals should put out to be totally diabetic are based on the usual calculations which of course are open to some question If there is gluconeogenesis from fat then we cannot say that these animals are totally diabetic

Markowitz It is a classical observation that the depancreatized dog puts out neither more nor less sugar when worked on the treadmill

On many occasions I have seen pancreatectomized dogs under strychnine convulsion excrete more sugar than at rest This is due to the lactic acid liberated and subsequently resynthesized to sugar No one that I know has claimed that muscular exercise in total diabetics dogs or humans, lessens the glucose excretion or blood sugar That does not mean your findings are incorrect but it justifies our questioning these things in this way

Ingle The only sort of observation that might be in line with this is that clinicians who manage diabetes believe that the insulin requirement is less when these diabetics take substantial amounts of exercise

Best There you have some insulin present That is what is bothering Dr Shorr Dr Markowitz and bothering me The picture is completely different in the total diabetic animal You cannot train them They cannot do much work, and the worst thing you can do for them is to make them work, but give them a little insulin and the more work they do, within reason, the better they are in sugar tolerance, training and everything else There is a sharp dividing line in the previous literature, but your work is much more careful than the previous work

Ingle I would admit the possibility that there may be some insulin left

Best If you have a trace of insulin the picture is entirely different

Gyorgy That is right

Shorr I wonder whether we should not entertain the possibility that the discrepancies between the depancreatized dog and alloxan diabetes as well as some of the phenomena seen under Dr Ingle s experimental conditions might be attributable to a parallel suppression of anterior

glycosuria to occur It is as if the alloxan animal in the basal state had small amounts of insulin present which could handle the basal carbohydrate need by virtue of a decreased ... uction of anti insulin factors by the pituitary or the pituitary c ... drenal corte

In this connection you may ... nding by ... and
Chandler of a progressive return o ... al type of ... te
metabolism in a few depancreatize ... happened ... for
15 20 days with the abolition of ... ketosis ... re
is no es ... explanation for t ... , the
that th ... stress resulted i ... f ant
diabet ... or in additio ... tion
change ... the so called ... d
to a pr ... this time t
fat towa

Attention may also be called to some of the metabolic characteristics of diabetic cardiac muscle Its R Q *in vitro* is in the neighborhood of 0 70, that of fat Yet at that R Q the turn over of high energy phosphates goes on at just about the same rate as in muscle from a normal dog burning at an R Q of 1 0 These observa tions, together with the extremely low glycogen values in the diabetic skeletal muscle, threw grave doubt on the necessity of diabetic skeletal muscle to depend on carbohydrate for energy

Ingle We have gone much further in activating muscle here than putting a dog on a treadmill I would certainly agree to the need for caution in assuming that these animals were entirely without insulin At certain times I have held that it is virtually impossible to be certain that one has rid an animal of any hormone This is particularly true of the sex hormones, and to know when every bit of adrenal tissue has been removed or when every bit of thyroid tissue has been removed is difficult I am quite certain that many hypophysectomies have not rid the animal of every cell capable of secreting pituitary hormones I sup pose that in the dog the removal of the pancreas can be really compl[illegible] It is not in the rat You can get pretty close to [illegible] you section everything [illegible] were destroy[illegible]

I was very [illegible] on rats which [illegible] thyroidectomized [illegible] to all of the usual criteria, had been completely [illegible] ago to hear of some studies the pictures were made of its location the experimenters were able to find accessory bits of thyroid tissue lying down in the chest Yet when radioactive iodine was administered and

Shorr Do you think that the effects of hypoglycemia were exerted on the muscle *per se* or via the nervous system? Were you able to per fuse the brain separately with a glucose solution do you think you would find that the muscle contractibility would persist? A number of years ago Cattell and I found that we could get prolonged contracti bility with skeletal muscle strips from the dog for long periods on electrical stimulation even in the absence of glucose from the medium Could impaired brain metabolism with hypoglycemia have been re sponsible for the muscle failure you observed?

Ingle I should think so Most of what I know about that subject was learned in the Mayo Institute When I was there Dr Corwin was working with Dr Bollman on the origin of hypoglycemic convulsions Those data indicated they were of nervous origin Has not considerable work been done in supporting that Dr Bollman?

Bollman Some not an awful lot though We would like to have more

On many occasions I have seen pancreatectomized dogs under strychnine convulsion excrete more sugar than at rest This is due to the lactic acid liberated and subsequently resynthesized to sugar No one that I know has claimed that muscular exercise in total diabetics, dogs or humans, lessens the glucose excretion or blood sugar That does not mean your findings are incorrect but it justifies our questioning these things in this way

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In this connection you may recall the finding by Chambers and Chandler of a progressive return of a more normal type of carbohydrate metabolism in a few depancreatized dogs which happened to survive for 15 20 days with the abolition of glycosuria and ketosis While there is no established explanation for this phenomenon, the most likely is that this metabolic stress resulted in a depression of anterior pituitary diabetogenic activity or in addition adrenal exhaustion These same changes occur during the so-called premortal phase in dogs subjected to a prolonged fast At this time their R Q 's rise from the level of fat towards unity

Attention may also be called to some of the metabolic characteristics of diabetic cardiac muscle Its R Q *in vivo* in the presence of glucose is in the neighborhood of 0 70 that of fat Yet at that R Q the turnover of high energy phosphates goes on at just about the same rate as in muscle from a normal dog burning at an R Q of 1 0 These observations together with the extremely low glycogen values in the diabetic skeletal muscle threw grave doubt on the necessity of diabetic skeletal muscle to depend on carbohydrate for energy

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I was very much interested a few months ago to hear of some studies on rats which according to all of the usual criteria had been completely thyroidectomized Yet when radioactive iodine was administered and the pictures were made of its location the experimenters were able to find accessory bits of thyroid tissue lying down in the chest

Shorr Do you think that the effects of hypoglycemia were exerted on the muscle *per se* or via the nervous system? Were you able to perfuse the brain separately with a glucose solution do you think you would find that the muscle contractibility would persist? A number of years ago Cattell and I found that we could get prolonged contractibility with skeletal muscle strips from the dog for long periods on electrical stimulation even in the absence of glucose from the medium Could impaired brain metabolism with hypoglycemia have been responsible for the muscle failure you observed?

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Bollman Some not an awful lot though We would like to have more

Ingle We would like very much to know what our contracting muscle can use other than glucose We know that if we work an intact fasting rat, stimulate only one muscle, it can work for periods of from five to eight days with the muscle contracting continually at the rate of three times per second During that time the other musculature of the body atrophies The working muscle sustains its size and certainly gains some weight Whether the weight is water or whether it is actually hypertrophy we don t know All of the fat stores of the body disappear during that time The animal can mobilize its fats for utilization in some manner Whether it is conversion to glucose we don t know We have tried to improve work performance with certain preparations of fat emulsions given intravenously It never works It only makes the rat sick and it dies

Best These that went five days, were they liverless?

Ingle No those were not I think I got through without noting our longest survival which is 65 hours *

Bollman That is just the same length of time as our liverless dog

Markowitz Did he have a transfusion?

Bollman That one had

Markowitz The comparison is not quite correct

Bollman It is not quite correct

Markowitz You gave some prothrombin?

Bollman He loses that very rapidly

Best I was interested in Dr Ingle letting his mind run along the channels that there might still be a few molecules of a suprarenal hormone after removal of the gland I think all of us are getting wedded to the idea We cannot imagine the body without our pet substance I confess I have often thought that the depancreatized animal when it loses the last molecule of insulin dies immediately afterwards but I know that is not true You can make glycogen *in vitro* I remember thinking of those experiments that Dr Mann did I don t know whether you were on that paper Dr Bollman or not on glycogen deposition of the liver in the depancreatized dog You made a struggle to make it without insulin You got a little I considered the possibility that a few molecules of insulin might still have been present Seriously I don t really believe there was any insulin

Bollman I wanted to make one comment I think we have gone a little bit afar I am sure Dr Ingle did not say the muscle was using the glucose in the test that he did for the blood sugar It is obvious that the glucose was disappearing from the entire animal not neces

* Now 82 hours and 48 minutes according to the most recent report from Dr Ingle—Editor

sarily that it was disappearing in the muscle which was being stimulated

Along that line, I wanted to ask you if you checked the heart rate on your stimulated and non stimulated rats Don't you get an increased heart rate with stimulation of the muscle?

Ingle Yes

Best Do you have a record of the heart rate on this device?

Ingle This is an all or none response We do have a little device though for measuring the heart rate in these rats

Bollman I think it is perfectly justifiable to make the inference that the muscle is doing something that causes the greater disappearance of sugar from the blood We are going a little afar to assume that the muscle is burning that sugar to do its work

Ingle I would certainly agree with that One of the other things that I dream about having some day is a perfusion setup which will permit us to isolate a leg and perfuse it with oxygenated blood with added nutrients, and if we could keep such a preparation contracting for a period of say as long as eight or ten hours that would be sufficient time to tell whether the adrenal hormones act in such a preparation and what insulin may be doing to it I think it would still be a complex biochemical system but it would be much more simple than having all of the rat

Bollman I have been impressed with the fact that most of the failures of muscle contraction by repeated stimulation have been related to circulatory failure rather than muscular failure As soon as you clamp the circulation to the muscle, the muscle is practically through At this rate of 3 or 5 stimulations per second it only has about a minute to go and then it is dead

Best Have you any data on the rate of disappearance of body fat with the standard amount of exercise in your liverless as compared to the intact animal?

Ingle No, I have not

Shorr There is one muscle that can be shown to utilize fatty acid *in vitro* This is the smooth muscle of the rabbit intestine If such a rabbit gut preparation is provided with no substrate, and washed once or twice with Ringer solution, the amplitude of its contractions progressively decreases When it has fallen to 5 10 percent of the original excursion, the addition of an effective substrate for the support of muscular contractility is promptly followed by an increase in the amplitude of the contractions Maximal contractions are achieved with glucose, and this substrate can serve as a standard Using this preparation Dr R F Furchgott and I studied a variety of substrates, including odd and even numbered fatty acids (Proc Soc Exp Biol & Med 61,

280 (1946)) Acetate had the same effect on amplitude as did glucose Even numbered fatty acid (4, 6 and 8 carbons) gave half the amplitude Odd numbered fatty acids (3, 5, 7 and 9 carbons) gave different but very interesting results Propionic acid did not restore contractions but actually inhibited the effect of butyric, apparently by competing with 2 carbon fragments for the surface of the enzyme systems involved As we went up the odd numbered series, increasing amplitudes were obtained, and with the 9 carbon pelargonic acid, the amplitude was almost as good as with butyric acid We felt that this was due to the increasing proportion of 2 to 3 carbon fragments The inhibitory effect of propionic acid on butyric could be overcome by adding appropriately greater amounts of butyric acid These experiments are taken to indicate that in smooth muscle, at any rate fatty acids can be utilized for contractile energy

Attempts by A V Hill, for example, working with the frog, to demonstrate a reduction in fat content of the contracting muscle were, I think, loaded against him because of the low energy metabolism of the frog muscle hence the small changes in fat content which could take place over the period of the experiment But I think it should be quite possible, using the mammalian muscle strip to determine directly whether fat is utilized

Best In smooth muscle?

Shorr In skeletal muscle

Best No one has ever demonstrated utilization of fat in skeletal muscle

Shorr No one has ever tried to, except for the balance studies of Hill on the frog I believe, however, that the conditions holding for the mammalian muscle strip of the dog, higher energy production, higher turnover, and the ability to contract for long periods of time—all these are favorable for a direct proof or disproof of fat utilization This experiment should be carried out because of its great interest

Markowitz No one has ever demonstrated it for the isolated heart perfused with Ringer s solution It is benefited by glucose and glucose alone It has been tried for acetates and propionate, succinates and fatty acids (which are difficult to do because you precipitate the calcium) but the fact is that an isolated beating heart does have some resemblance to skeletal muscle, and the only thing which does it any good is glucose

Shorr However, fat utilization has been shown to take place in the isolated heart lung preparation of the diabetic dog by Cruikshank and Startup (J Physiol *81*, 153 (1934)) In the absence of insulin, the respiratory quotients were always in the neighborhood of fat 0 70

When insulin was added to the perfused blood, the R Q rose to unity The differences between these results and those just cited by Dr Markowitz may arise from difficulties presented by isolated heart perfusion experiments using Ringer solution The addition of fatty acids to a Ringer solution may not present this material to the heart in a physiological state We observed for example that when fat was extracted from blood by alcohol and ether and added to the rabbit intestinal strip, contractions were inhibited This inhibition could be overcome by the addition of serum albumin Whatever the cause, I am inclined to accept the work of Cruikshank and Startup, because of its more physiological character and the care with which it was carried out

Armstrong Does not the isolated heart utilize ketone bodies?

Markowitz It does utilize ketone bodies I am talking about Ringer s solution There is no blood in that I refer to the effect of Ringer s solution alone, on the rabbit heart, the only thing that benefits that heart to my knowledge—and I am very much open to correction—is glucose Whether ketone bodies can be used or not I would not debate They probably are used to benefit the heart It would not live any longer by the addition of ketone bodies To keep a surviving heart beating for long, one must add glucose and nothing else is of any use

Shorr Don t you think, Dr Markowitz that this problem is complicated by the metabolic peculiarities of heart muscle?

Markowitz No

Shorr I d like to cite from my own studies certain striking differences between diabetic skeletal and cardiac muscle as indicative of metabolic differences between these two tissues (Cold Spring Harbor Symposia on Quantitative Biology, 7 323 (1939)) Although the respiratory quotients with both tissues are usually in the neighborhood of 0 70, there is a significant difference in their ability to glycolyze The skeletal muscle forms very little lactic acid under anaerobic conditions whether or not glucose is in the medium In contrast, cardiac muscle has a high rate of glycolysis which is significantly increased by the addition of glucose This difference appears to reflect in large measure the extremely low glycogen content of skeletal muscle in the diabetic dog and the high and indeed greater than normal content of the cardiac muscle from such an animal (N F Fisher and R W Lackey, Am. J Physiol 72, 43 (1925)) The total fermentable carbohydrate in diabetic heart is likewise greater than normal in our experiments averaging 13 5 mg per gm as compared with 7 9 mg per gm in the normal controls These high glycogen and total fermentable carbohydrate values for the diabetic heart indicates that this tissue in contrast with skeletal muscle, retains the capacity to store glycogen in the absence of insulin even

though it cannot oxidize glucose without the help of this hormone Apparently the carbohydrate metabolism of the various tissues is not equally affected in diabetes Indeed the brain can oxidize glucose without insulin

Markowitz If blood is used from depancreatized dogs only, and a heart lung preparation is made from a depancreatized dog that heart survives an hour or two and nothing you can do will make it survive longer unless you add insulin

Best There is glucose

Markowitz This is a heart lung preparation with sugar in the blood but without insulin There must be some difference between diabetic muscle and non diabetic assuming that a heart muscle is a fair test object Those facts have never been disproven Knowlton and Starling made them I have been able to repeat them and my results were so entirely uniform I did not bother to report them The diabetic heart perfused with diabetic blood does not survive much longer than an hour

Best The amount of glycogen in the diabetic heart as compared to a normal heart is not very different

Shorr The figures I cited above for total carbohydrate were about 30 percent higher in the diabetic heart than in the normal I think the important thing is that the glycogen content is at least normal whereas the glycogen content of the diabetic skeletal muscle is negligible

SECTION VI

LIVER GLYCOGEN AND THE PROTECTION OF LIVER CELLS

Best I think many of us who are interested in the protection of the liver found our allegiance divided between protein and perhaps methionine and choline on the one hand and the storage of glycogen, with or without insulin on the other I was quite sure that I could not do justice to the carbohydrate side of it and I was anxious to have the field reviewed so I thought it would be a good idea to invite Dr Bollman to review it completely and tell us everything about it

He has reacted a little bit to this He is not going to cover everything today He is going to take part of the hour to tell us about work on fat absorption

Bollman Had I been able to find in the literature or perform experiments myself which would furnish direct evidence and absolute proof that the resistance of the liver to hepatic toxic agents varies directly with the carbohydrate content of the diet or the glycogen content of the liver, I could give a short and satisfactory exposition Instead I will have to talk around the subject

In terms of the daily caloric need of the body, the storage space for carbohydrate is limited and failing a source of supply of glucose for blood sugar as in the hepatectomized animal, life soon terminates The liver is able to fabricate glucose from non carbohydrate sources but such processes are accomplished only with the expenditure of energy The diet of all animals contains carbohydrate at least in concentrations equivalent to the proportion of carbohydrate contained in their body Most mammals including man consume a large excess of carbohydrate in relation to the composition of the body which contains (man) about 45 times as much protein as it does carbohydrate (H H Mitchell, T S Hamilton, F R Steggerda and H W Bean, J Biol Chem *158* 625 (1945)) Carbohydrate is known to spare protein and will reduce the nitrogen loss in the urine when fed to otherwise fasting animals Carbohydrate fed with protein will also allow a greater protein storage than when protein is fed alone In rats fed on a high carbohydrate diet sufficient to maintain their weight, isotope studies indicated that about 69 percent of the liver glycogen was replaced daily (DeW Stetten Jr and J E Boxer J Biol Chem *155*, 231 (1944))

Protection to the liver by carbohydrate may be accomplished in two ways, one in providing glucose for the maintenance of the entire body at no expense to the liver and second, by sparing protein which may

be used for rebuilding of the damaged liver and for other purposes Many clinical observations of liver diseases and also observations on experimental animals following surgical damage of the liver *i e* partial hepatectomy Eck s fistula biliary obstruction etc indicate better survival rates when carbohydrate is given Whether the glucose or carbohydrate administration had any direct effect in sparing the liver or merely introduced factors which maintained life long enough for the liver to repair itself cannot be ascertained I am sure that many other factors are present and that life cannot be maintained indefinitely on glucose alone Certainly all the other food factors must also be supplied if the animal is to live and especially so if he has to repair a liver injury We know

carbohydrate to

any measurable

amino acids of the diets more rich in protein are converted to urea by the liver and excreted in the urine and with diets relatively poor in protein the amino acids are conserved so that the actual protein content of the body is the same Since the liver is involved in both the destruction and the conservation of amino acids it is probable that the diseased or injured liver would not have the flexibility of a normal liver and that recovery and repair would be expedited more by supplying the liver with glucose and amino acids at some specific ratio which may not be as flexible as the normal dietary ratio

I want to call attention to the constantly changing composition of the liver and its extreme flexibility in that it seems to function well over a wide range of chemical composition (Slide) This slide shows the changes in liver weight and glycogen protein and fat content of the liver following a meal Rats were trained to eat a full meal in one hour and on the day of the experiment were fed a diet containing 70% carbohydrate 20% protein and 10% fat at 9 00 a m This is indicated as time 0 on the slide and groups of rats were taken at subsequent intervals over the 24 hour period for analysis After feeding there is an increase of about 20% in the weight of the liver by about the 4th hour and a subsequent return to the original size in about 12 hours The glycogen content changes even more after the food is absorbed and later there is a decline below the fasting level with a return within 24 hours to the original fasting level The protein and fat content of the liver change in the same way but the changes are less extensive

This second slide is an example of the thing that I am not supposed to say that carbohydrate definitely can injure the liver

Best That is the reverse of what you are supposed to say

Bollman That is the reverse of what I want to say I want to bring it out for this reason These sections were taken from a dog who had

received continuous intravenous injection of glucose for about 36 hours The amount of glucose was so given that he always had a high blood sugar, somewhere between 200 and 300 mg per 100 cubic centimeters The dog was excreting a large amount of sugar The dog received about 3 grams of glucose per kilo per hour In the meantime the liver glycogen did accumulate At the time these sections were taken, after 36 hours of continuous infusions the liver was full of glycogen, the dog had a very definite bilirubinemia and a very marked dye retention This is ample proof, I believe, that carbohydrate can injure the liver

Hanger You think the functional impairment is secondary to circulatory disturbances?

Bollman I have no real idea as to the mechanism of the injury It would seem from the examination of the liver that it is so full of glycogen it has neither time nor ability to do anything else This is obviously an excessive strain and is not the particular thing we were talking about

Best You know of the active debate now going on in clinical circles as to the use of dextrose in the treatment of diabetic coma, one school asserting that the use of very large amounts of dextrose intravenously may kill the patient?

Bollman Yes

Gjorgy Do you agree?

Best I don t agree, no, without reservations I think that is another subject

Watson From liver injury It was more related to the potassium situation and adrenal function

Best Howard Root at the Academy one night talked of liver injury

Gyorgy From high glucose?

Best In the treatment of diabetic coma

Gyorgy The Boston group emphasizes the potassium deficiency

Best I think Root at that time had some specimens from animals to illustrate his talk

Bollman I don t believe that any clinician would be idiotic enough to inject three grams of glucose per kg each hour for 36 hours That would mean the injection of about 40 liters of 10 percent glucose a day

Watson I was thinking about a big excess I won t say 3 grams per kilo per hour In a number of places they are giving 20 percent glucose intravenously with an inlying plastic tube giving it day after day

Best With vitamins?

Watson They give a lot of vitamins

Best If you are losing a lot of sugar in the urine anyway it does not

make much difference whether you are giving three grams, four grams or two grams Some individuals eliminate more and others less

Bollman That is right

Neefe Do you think that the bilirubinemia and the dye retention could be the result of a competition of functions?

Bollman It is a failure of those functions Let me hasten to add that this is not a permanent injury The next day those animals would recover and the glycogen will fall fairly rapidly if you discontinue the injection You can, I should say, injure the liver by carbohydrate I also should add at the same time this glucose was given with about 2 per cent saline, but no other inorganic salts, and necessarily a large volume of fluid We washed a lot of things out Whether the liver injury was due to the things we washed out or the things we washed in it is still a question Of course that same thing comes up in clinical cases that you are doing two things You are putting some things in and also washing out some things, unless you use a perfectly balanced solution I know of no such solution

(Slide) This represents a fatty liver which can be produced by reducing the protein of the diet This was from a dog that received 90 percent of his calories in the form of fat In about 8 weeks such dogs develop a fatty liver That fatty liver had perfectly good function as far as we could tell There was no dye retention

Best What amount of fat is that?

Bollman That one was about 22 percent My experience with staining suggests that one can make a fairly good guess as to the fat content in the dog liver when it increases from about four percent to eight or nine percent After it gets beyond eight or nine percent, an accurate estimation is impossible

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EDITOR Dr Bollman next reviewed certain data previously published (see Transactions of the Fifth Conference on Liver Injury, Pages 11 to 14 1946)

[illegible] ch rats arc [illegible] and a spi[illegible] *adding* oxygen to the spirometer and taking the CO_2 out with a CO absorber, we have essentially a Benedict table We can put our rats in the chamber and expose them to air plus any noxious agent that we wish to add to the air We may also take away the oxygen if we wish

We have used this particular setup in two series of experiments so far One was to test the effect of ether with reference to the survival of the rats We put in the ether by recirculation and maintained the concentration at a level at which 50 percent of our well fed normal

rats would die within a period of 4 hours Then we tested a group of rats that had been fasted for four or five days and found that they succumbed at exactly the same concentration of ether as did the normal and well fed rat In other words, our feeding gave us absolutely no protection against this same concentration of ether That perhaps is not surprising but at any rate there was very definite evidence that the well fed rat was no better, as far as the ether exposure, than was the fasting rat

Gyorgy What did they die from?

Bollman The ether concentration

Gyorgy It was not necessarily a liver death?

Bollman No that is not necessarily a liver death by any means We have also done another series of experiments testing the effect of anoxia By merely replacing the oxygen with nitrogen, we dropped the oxygen content down to about 5 percent The rats would succumb in about half an hour At a 50 percent level, they seem to be able to accommodate to this and will live five or six hours However, at a very low oxygen content we found no difference between fasted rats and the fed rats In a rather small series we injected glucose immediately before exposing them to this period of anoxia and again there was no difference in the survival The ability of the rat to withstand the anoxia certainly is not a function of the liver entirely but the liver is certainly involved The anoxic rats have livers that are nearly black Such livers are large and swollen and certainly appear anoxic

Shorr Is this a 24 hour fast?

Bollman No a longer period

Shorr Four days?

Bollman The results after 24 hours were negative That is the reason we went to a longer period of time

To get back to the original story, I think that we are all convinced that carbohydrate is very valuable food and does offer protection to the entire body and to the liver

Best You have not found any evidence that that is so?

Bollman We have no direct evidence that that is true Pavlov and Root were the first to demonstrate in 1890, that the Eck fistula animals survived much better without meat and on a high carbohydrate diet than on a high protein diet That is not proof but clinical observation We have clinical observations on postoperative dogs where the liver is involved It appears that with very severe injury to the liver, we can better the animal s chances of surviving if we give it glucose and maintain the animal on a high carbohydrate diet than if we omit glucose, and give them a high protein diet That is marked We have some

examples of that Let us take for example the dog that has had his common bile duct ligated for about three months Such an animal is not a sturdy specimen If we put such dogs on a diet composed entirely of *meat—not* a bad diet for a dog—they usually die in less than a week If we give them a low protein, high carbohydrate diet consisting not entirely of carbohydrate by any means but containing protein and other food factors, we keep those animals alive for many months almost a year They usually develop a perforated duodenal ulcer and die

Best What about the caloric value of the food that they are able to absorb in the two cases? If you tie the bile duct you get very little absorption of fat, I take it

Bollman I really don t know just exactly what the caloric value of the food that they absorb would be I don t believe that that is a factor because we are talking about a week as compared to several months

Markowitz Do they get ascites?

Bollman They do

Hanger You say these animals develop ascites in contrast to others?

Bollman The meat fed animals develop ascites

Gyorgy They have only meat, nothing but meat?

Bollman Yes

Best Meat extract will do that

Bollman It is not protein It is meat, and meat extract will do the same thing, so I don t believe that the protein has anything to do with that.

Hanger What is the mechanism of the ascites?

Bollman I really don t know

Gyorgy Cirrhosis of the liver

Bollman Biliary cirrhosis, yes

Patek Would that be true of cooked meat as well as raw meat?

Bollman Yes

Watson Don t you think that must be a species difference? Have any of your clinicians ever cited any clear cut example of that in human cirrhosis? I have watched for that for years and I have not seen it We feed such patients a lot of meat

Bollman Let me add one other additional qualifying statement this particular development of ascites is under a very special set of circum stances The dogs have to have biliary obstruction for three months I have never seen it in less than three months They have a certain amount of liver damage, ascites will also occur if we continue these animals without meat for sooner or later they will develop ascites spontaneously

Best Isn t this the same story exactly as in Eck fistula dogs?

Bollman The same thing I think, and that is as much evidence—I hate to admit it—as I could find of the protective action of carbo hydrate on the liver

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Gyorgy What was the relationship in the case of fat?

Bollman The idea that glycogen is reciprocal of fat in the liver is also erroneous because one can have very high glycogen present in a very fatty liver

Best There are limits there?

Bollman There are limits

Best You cannot have a lot of glycogen in a very fatty liver for example 40 percent or even 30 percent You don t get glycogen in a very fatty liver You cannot have a lot of fat in a liver that is very rich in glycogen but in the intermediate stages you can in both cases

Bollman In the intermediate stages yes At the extremes of course one is dealing with something else again

Patek Is it possible that the protective effect of carbohydrate in certain instances might be due to a protein sparing effect?

Best I don t know whether you should spare the protein

Patek If one employed two series of rats on weighed diets with the same low protein content the remaining calories made up with carbo hydrate in one group as against fat in the second group it might well be that the high carbohydrate diet would be more readily tolerated because of a protein sparing effect

Best I think if I were going to go at this problem I would start with the resistance of the untreated diabetic animal to liver poisons

Gyorgy For the fatty liver? They have a fatty liver

Best Yes they have a fatty liver but they go on to get cirrhosis The untreated diabetic animal will get cirrhosis if he just lives long enough In the old days the diabetics got cirrhosis not the severe diabetics since they died but the mild diabetics frequently got cirrhosis I am talking of human beings

Bollman One of the reasons I accepted this assignment was not the fact that there was anything particular to say but I would like some suggestions to find out how we could check this

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Best I think almost any clinician would say that the untreated diabetic was more susceptible to a lot of toxic influences than the well treated one One difference between the two is that one has liver glycogen and the other has not

Gyorgy Or one has fat and the other has no fat

Best There are lots of other variables

Bollman They do not make very good experimental animals I have tried that and I have tried the Eck fistula animals thinking that I could use alcohol or carbon tetrachloride The only trouble is that with that their susceptibility increased, all you have to do is blow a little chloroform at them and they turn up their toes and die

Best You get a dramatic difference I should think between a treated diabetic and an untreated diabetic

Bollman Of course

Best You cannot say that this is due to depletion of liver glycogen or to an increase of fat

Bollman That I think is very definite, there again is some indication of the value of carbohydrate

Best I am not sure that it is I am not sure except that I believe you would have no difficulty in showing that with more or less specific hepatotoxins the insulin treated diabetic animal would survive more frequently than the untreated

Bollman I thought everybody knew that

Best Do they? I take it for granted that this would happen but I don t know where to find the data in the literature

Bollman I tried it in dogs I never published it

Best You should because it would be very valuable, perhaps more than anything we have unearthed today

Bollman Thank you!

Best I would not have undertaken this problem I am very grateful to Dr Bollman for doing it

Watson There are features from the clinical side which I think ought to be mentioned I would like to hear just a little bit of discussion about the effect of glucose in the patient with severe liver disease

I had a most remarkable experience a year ago The patient was an alcoholic She had severe cirrhosis of the liver She came into the hospital with ascites and edema We started her in on a high protein intake I think she was getting about 150 grams a day, part of which was casein and some meat We had some difficulty feeding her She was taking it reasonably well but she did not improve She persisted in having ascites and edema and had quite a bit of jaundice and many

evidences of liver dysfunction We then gave her human albumin intravenously We gave about 900 grams in a period of about 12 days and go the serum albumin up to 5 2 grams percent but there was no diuresis In fact she had increasing oliguria of a mild degree and gained weight about ten pounds and her ascites increased We did not tap her dry at the outset Perhaps we could have gotten a better effect had we done so but in any event she got worse during this period We were getting in quite a lot of protein during this period as we had given 900 grams of alubumin intravenously in addition to the high protein diet Nevertheless she became semi comatose and everyone who was watching her thought she was going to die At that time we obtained a liver biopsy This was remarkable in that there was still a good deal of fat in her liver It was a severe cirrhosis and there were many spaces that were just empty which appeared to be the result of liver degeneration There was no necrosis but considerable fibrosis and a great deal of cellular exudate We were at our wits ends and decided we would give her glucose She received 20 percent glucose intravenously in amounts of 300 grams a day She promptly improved and made a remarkable recovery She had a good diuresis and lost weight At present she is quite well and without jaundice ascites or edema I realize that she may have had a delayed benefit from the albumin If so it was delayed about 15 days During the period of albumin and high protein therapy not only did she not benefit but visibly she got worse

Gyorgy Did you give injections of vitamins all the time?

Watson No

Gyorgy I am very much interested in that that is rather a common practice in many clinics

Watson We don t often and I don t believe we did here

Best Did you stop the intravenous protein when you gave the sugar?

Watson Yes the albumin was stopped for about 48 hours an interval of just marking time trying to decide what to do

Best Was the essential amino acid composition low in methionine?

Watson Of the albumin? I would say it is good

Gyorgy It is lower in isoleucine according to Fenster

Watson The patient had a pretty good general protein intake by mouth in addition to the albumin intravenously I cite that case not in any attempt to say that glucose was the thing that saved her life but simply to show at least that glucose did her no harm She got 300 grams of glucose a day at a time when she was in coma I have seen that again and again I have seen a number of patients in hepatic coma at least come out of their coma when they got a lot of glucose intravenously so that I think we can say at the least that the glucose probably

isn't doing them any harm Whether it is the thing that is bringing them out of coma I would hesitate to say because we do do other things We do give them vitamins in varying degree

Gyorgy In coma especially, we are fond of niacin Dr Neefe did you not find in a few cases at least an indication for what we call the vitamin cocktail?

Neefe I think that originated at the Mayo Clinic They gave large doses of nicotinic acid and thiamine and had dramatic responses I don't know whether they gave glucose or not I think that they did

Patek Dr Chester Jones has maintained for years that infusions of glucose would produce remissions in subacute hepatitis and in the pre-chronic phases of cirrhosis

Hanger We make no effort to keep up the protein intake during an episode of acute hepatic insufficiency We rely chiefly upon glucose by slow infusion

Best It seems to me that there is very convincing clinical evidence that the glucose does a great deal of good but that the experimentalists have completely failed to find any reason for this

Bollman That is correct

Best Had Dr Stetten been here he would have given us a little talk on liver glycogen as a thing as obsolete as the vermiform appendix

Gyorgy That is right a vestige

Best Yet when you give the glucose you probably do get more liver glycogen A lot of other things also happen so perhaps we have been wrong in just looking at liver glycogen to find the explanation of a beneficial effect of glucose

Watson What observation would you suggest to the clinician who is going to be able to study just such a case as I mentioned? What other thing should one look at to try to find out how glucose is beneficial if it is?

Best Study it in the untreated diabetic

Watson I was thinking of the patient with hepatic coma or pre coma What observations could one make to settle the question as to whether the glucose was having a rather precise effect?

Best My thoughts are more in the experimental field where you have to eliminate certain organs to get a clear answer

Hanger Why do you think they come out of coma?

Markowitz It might be circulatory effect Three hundred grams of glucose over the course of a day is bound to improve the blood flow, shall we say to the liver The blood is going around perhaps twice as fast while you are giving it

Best You spoke of the effect on the heart?

Markowitz That effect, sir, is only shown when no glucose whatever is in the infusion The addition of a trace of glucose is about as good as the addition of a gram

Gyorgy It may be the niacin and thiamine

Watson The effect of the glucose on the brain might be very important because in hepatic coma there is brain injury You can see it with the microscope

Knisely If you are going to have glucose bringing the patient out of coma, it has to go to the brain

Markowitz For twenty five years I have been treating patients with liver disease with intravenous glucose I hate to think it accomplished nothing

Best I don t think there is any intent that our lack of experimental information should discourage the clinicians in the use of glucose We should be stimulated to find some better reason, some better explanation We really have never set up the proper experiments

SECTION VII

THE LOCUS OF THE BEGINNING OF DIETARY CIRRHOSIS

Hartroft Evidence has been presented by L L Ashburn, R M Endicott, F S Daft and R O Lillie (Am J Path 23, 159 (1947)) to show that the distribution of the fibrous trabeculae is centrolobular This has been supported by L E Glynn, H P Himsworth and O Lindan (Brit J Exp Path 29, 1, (1948)) Recently this has also been accepted by P Gyorgy and H Goldblatt (J Exp Med 89, 245 (1949)), who originally (Ibid 70, 185 (1939) and 75, 355 (1942)) considered the fibrosis to be periportal Yet many other investigators[1], who have described the microscopic appearance in this type of cirrhosis, indicated that the fibrous tissue surrounded the portal canals At one time, we also thought that possibly the distribution of fibrous tissue in the livers of our choline deficient rats might be portal, although it was stipulated that further evidence was needed The observations which follow were made for the purpose of obtaining additional information concerning this problem The results indicate that, rather than portal, the fibrosis appears to be definitely central in distribution The question is perhaps somewhat clarified, however, since some of the observations indicate a possible explanation for the divergent conclusions which have been reported in the interpretation of the microscopic features of this type of cirrhosis

Some investigators (C L Connor and I L Chaikoff, Soc Exp Biol and Med 39, 356 (1938)), L E Glynn, H P Himsworth and O Lindan (Brit J Exp Path 29, 1, (1948)) and others, support the view that the fatty infiltration of the liver which precedes dietary cirrhosis may be a primary etiological factor in stimulating fibrosis Others (P Handler and I N Dubin, J Nutrition 31, 141 (1946)), (P Gyorgy and H Goldblatt, J Exp Med 89 245 (1949)) question this concept The observations to be described below, appear to give further support to the view of Connor and Chaikoff, Glynn *et al* Some of the findings also suggest a possible route by which the excess fat might escape from the liver during the later stages in the development of cirrhosis These

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[1] I L Chaikoff C L Connor and S R Biskind (Am J Path 14 101 (1938) depancreatized dogs) C L Connor and I L Chaikoff (Proc Soc Exp Biol & Med 39, 356 (1938) normal dogs fed alcohol and high fat diets) I L Chaikoff and C L Connor (Proc Soc Exp Biol & Med 43 638 (1940) normal dogs fed high fat diets alone) A R Rich and J D Hamilton (Bull Johns Hopkins Hosp 66 185 (1940) rabbits fed diets deficient in yeast) G Webster (J Clin Investig 20 440 (1941) 21 385 (1942) rats fed low protein diets) H Blumberg and H S Grady (Arch Path 34 1035 (1942), rats fed low protein diets) R L Holman (J Exp Med 81 399 (1945), dogs bled while fed on high fat diets)

aspects of the pathogenesis of dietary cirrhosis will now be presented in detail

INVESTIGATIONS CONCERNING THE PATHOGENESIS OF DIETARY CIRRHOSIS IN CHOLINE DEFICIENT RATS

Methods

A diet deficient in choline and its precursors[2] was fed *ad lib* for periods up to seven months to 120 young adult male rats (150 grams) of the Wistar strain This food mixture was relatively deficient in tocopherol At intervals separated by 24 hours during the first ten days, and twice weekly during the following three months of the experiment, two animals were selected at random and sacrificed During the remainder of the experimental period, groups of five animals were killed at monthly intervals Under sodium amytal anesthesia[3], the hepatic vascular system in the heparinized animal after perfusion with normal saline, was injected under physiological pressure *via* the beating left ventricle with a filtered, diluted colloidal suspension of carbon[4] Blocks of tissue selected from the right and the left lobes of the liver were

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[2] DIET

Arachin	12%	Beef fat	10%
Gelatin	6%	Corn oil	2%
Casein	3%	Cod liver oil concentrate	0 015%
Fibrin	1%	Cystine	0 50%
Salts	5%		
Celluflour	2%		
Sucrose	57 5%		
Vitamin powder	1%		

The composition of the salts used in the above per 100 gms was

Calcium lactate	35 0 gms.
Calcium carbonate	5 06 gms
$Ca(H_2PO_4)_2 H_2O$	14 60 gms
K_2HPO_4	6 46 gms
$NaH_2PO_4 H_2O$	18 76 gms
NaCl	9 34 gms
$MgSO_4$ (anhydrous)	7 18 gms
Fe citrate	3 20 gms
and trace salts	
$MnSO_4 4H_2O$	0 33 gms
$ZnSO_4 7H_2O$	0 035 gms
$CuSO_4 3H_2O$	0 039 gms
KI	0 00039 gms

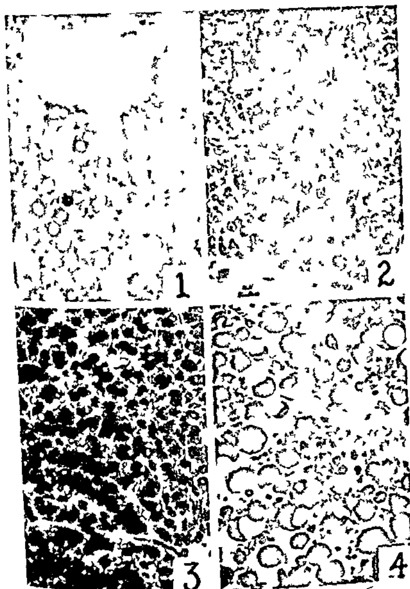
The Vitamin powder per 1000 gms of mixture contained

Thiamin	500 mgm	Riboflavin	250 mgms
Pyridoxine	200 mgms	Calcium pantothenate	1 gm
Nicotinic acid	1 gm	and powdered sugar	997 05 gms

The cod liver oil concentrate used (C L O conc) contained 50 000 I U of Vit D and 200 000 I U of Vit A per gram (Supplied by Messrs Ayerst McKenna and Harrison Montreal)

[3] 90 mg of sodium amytal per kilogram of body weight administered intraperitoneally as a freshly prepared 10% solution in distilled water

[4] Higgins American India ink diluted with two parts of distilled water A few drops of ammonia added to the mixture ensures suspension of the carbon particles


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that 30 to 40 percent of the wet weight of the liver is lipid (L H Ridout, C C Lucas J M Patterson and C H Best, Science *103*, 12 (1946))

Fatty livers produced in rats by one week of choline deficiency rapidly revert to normal when lipotropic factors are restored to the diet Within 24 to 48 hours, the cells at the periphery of the liver lobule are completely devoid of stainable fat (Figs 5 and 6) Centrolobular lipid can still be seen, but this is mostly in the form of several small droplets instead of large spherules After three or four days on the normal diet, it is difficult to find any stainable fat throughout the sections (Fig 7) Liver cells which have been rapidly depleted of sudanophilic lipid in this manner, frequently give pale staining reactions and have a smooth, glassy type of cytoplasm

These observations indicate that the cells in the central portion of the liver lobule are not only the first which become affected by fatty change in dietary choline deficiency, but are also the last to return to normal when lipotropic factors are restored to the food

Circulatory Changes in the Fatty Liver

In sections of livers which contain a high percentage of lipid, the degree of distension produced in parenchymal cells by large vacuoles of stainable fat is great (Fig 8) The cells have enlarged at the expense of the intervening sinusoids In routine sections the intralobular vascular channels are inconspicuous and difficult to demonstrate The lumina are often so small that their positions are only indicated by the flattened linings of endothelial cells

Cleared liver slices (100 μ), from rats injected intravenously with

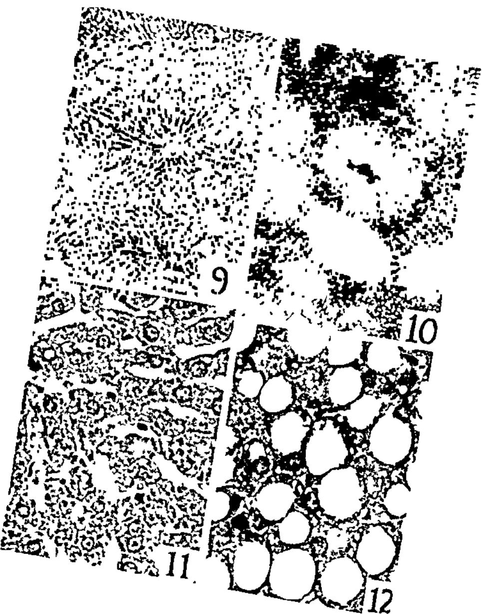
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FIGURE 9 Normal liver from a control animal which has been injected with India Ink *via* the hepatic artery and portal vein The tissue was subsequently fixed in formalin and a thick (100 μ) slice cut on the freezing microtome The slice was then dehydrated in alcohol and cleared in benzyl benzoate Note the appearance of the normal intralobular sinusoids x 50

FIGURE 10 The appearance of a very fatty liver from a rat fed a choline deficient diet for 7 days which received the same treatment as the preparation shown in Figure 9 Note the compression of sinusoids in the central and mid portion of the lobules Ink in the central and mid portion of the lobules Ink in the central veins demonstrates that the ischaemia was not complete x 50

FIGURE 11 The size and appearance of liver cells in a paraffin section of a normal control rat (150 200 grams) This is for comparison particularly with Figures 12 13 and 14 which have received the same photographic technic, and are at exactly the same magnification Paraffin section McGregor stain (azocarmine anilin blue and orange G) x 860

FIGURE 12 Fatty vacuolation of individual liver cells in a rat twelve days of dietary choline deficiency The diameters of these cells less than twice those of normal ones Paraffin section McGregor


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ink, facilitate adequate visualization of the intralobular vessels (Fig 9 & 10) An appreciable decrease in the diameters of centrolobular sinusoids is demonstrable in fatty livers Since the injection mass which came to the liver *via* the portal vein and hepatic artery, reached the central veins, the centrolobular ischaemia can only have been partial Had it been *complete necrosis of the surrounding* parenchyma would have resulted The injected preparations indicate that there is a diminished blood flow in the central portions of the lobules This appears to be due to sinusoidal narrowing produced by pressure exerted on the channels from without by the swollen, fatty parenchyma It is not surprising that the effect of the extra sinusoidal pressure is most apparent toward the center of each lobule, for here the intra sinusoidal pressure is lower than at the periphery and the vessels are more readily compressed A description of a similar type of circulatory impairment has been given by L E Glynn and H P Himsworth (Clin Science, 6, 235 (1948)) following hydropic swelling of cells in the centrolobular *regions of livers of rats treated with carbon tetrachloride*

Lipodiastaemata, Their Concept Structure and Formation

Microscopic evidence of fibrous tissue proliferation in these livers was not infrequently observed in rats fed the basal diet for periods as short as 8 weeks In one case new fibroblasts were found after the animal had been fed the choline deficient diet only 6 weeks In the stage of early hepatic fibrosis, the spaces occupied by stainable fat achieve

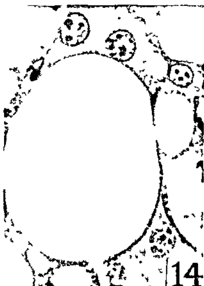
FIGURE 13 The large space occupying half of the field is a medium sized lipodiastaema (see text page 136) Note the very thin septum separating the two cells in the other portion of the picture If this septum should rupture, another lipodiastaema would be produced equal in size to that shown below The very black, round or oval spaces represent injected ink in sinusoids From an animal fed the deficient diet for 45 days Paraffin section of liver McGregor stain x 860

FIGURE 14 A giant lipodiastaema is shown Two nuclei of cells forming its wall are present at the right The diameter of this space is 7 8 times that of a normal liver cell (volume 350 400 times greater than normal liver cell) The liver cell nuclei have also increased in size but the small endothelial cells afford an *index of comparative size From a rat fed the deficient diet* 51 days Paraffin section of liver McGregor stain x 860

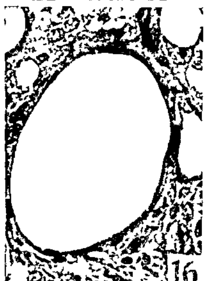
FIGURE 15 The lipodiastaema in the upper left portion possesses an outline resembling that of an hour glass This suggests that the lipodiastaema has just been formed from two smaller spaces by rupture of a septum between them which has incompletely retracted Paraffin section of liver of a rat fed a choline-deficient diet 65 days McGregor stain x 860

FIGURE 16 A lipodiastaema with several compressed nuclei in the wall A fine fibril of connective tissue surrounds the structure Some of the flattened nuclei may belong to narrowed sinusoids around the lipodiastaema Liver *of a rat after* 65 days of choline deficiency Paraffin section McGregor stain x 860


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relatively enormous proportions Parenchymal cells in paraffin sections of livers of control rats averaged about 14 μ in diameter (Fig 11) In livers of animals fed the choline deficient diet for twelve days, the diameters of the fatty cells seen in sections were increased, measuring 18 20 μ (Fig 12) After longer periods (45 and 51 days in the cases of the animals from which the sections illustrated in Figures 13 and 14 respectively, were prepared) the spaces occupied by fat were found to have diameters measuring as much as 100 μ or more in the paraffin sections This is greater than 7 times the diameters of liver cells in sections of normal rats, it is an astonishing fact that the volume of these large spaces must therefore be 350-400 times that of a normal hepatic cell The total liver weight, however, is increased to only two to three times the normal (J H Ridout, C C Lucas, J M Patterson and C H Best, Science, *103*, 12 (1946)) This strongly suggests that these large spaces no longer represent the accumulation of stainable lipids in just one liver cell as in the earlier stages

As vacuole is the term commonly employed when referring to the stainable fat of a single cell, it is not applicable to these giant spaces The name lipodiastaema (lipo—fat, diastaema—space between) is suggested for the latter Evidence will now be given which suggests that the fusion of several fat vacuoles, each originally present in a single cell, produces a lipodiastaema

[illegible] weeks, large [illegible] affin sec [illegible] is always [illegible] a fraction of the total number of cells attained the size of a lipodiastaema, the liver would have a volume much greater than only double or triple the normal As has already been stated, this is not the case This evidence suggests that several cells probably take part in the formation of a lipodiastaema If this is so each lipodiastaema should contain several nuclei This appears to be true In thin (4 μ) sections usually at least one nucleus (Fig 13) and frequently two (Fig 14) are seen This in [illegible] one nucleus

In paraffin sections the adjacent limiting membranes of two contiguous liver cells swollen by fat often form partitions between neighbouring vacuoles These partitions are so stretched and thinned that even under the highest powers only a single, tenuous membrane can be resolved (Fig 13) Frequently these delicate partitions are broken in

the sections and their severed ends separated by space These membranes are either ruptured during life or they are torn during the handling and preparation of the tissue They are either fact or artefact Many such breaks doubtless are produced by the sweep of the microtome knife despite the support supplied these membranes by paraffin However, lipodiastaemata in sections may sometimes have outlines which resemble those of hour glasses (Fig 15) This suggests that rupture of a septum originally separating fat vacuoles of two liver cells had occurred sufficiently long before the animal s death to permit incomplete retraction of the free ends of the severed membrane When the membranes are so stretched and thinned that they are easily torn by the microtome knife, this *post mortem* occurrence may be viewed in one sense as merely accomplishing a result which might have developed in the animals had death not supervened

Having introduced the concept of lipodiastaemata and presented evidence for their existence in fatty livers, their structure and formation will be considered in more detail As indicated, a lipodiastaema results from the rupture of a thin membrane which becomes stretched and compressed by expanding fat vacuoles which distend adjacent liver cells Dissolution of the membrane which had previously kept the fat of these cells in separate globules, allows them to coalesce into a single mass In this form, the fat is now contained in the lumen of an unilocular sac formed by the conjoined parent cells These are the cells, each of which previously contained a portion of the fat now in the form of a single unit Thus the lipodiastaema possesses a wall consisting of a continuous, single layer of thin, epithelial cells which surrounds a lumen filled with lipid The size of each lipodiastaema and the number of nuclei present in its wall will depend on the number of liver cells which at any time have contributed to its formation At the time of its formation, the smallest lipodiastaema would have a volume at least twice that of single liver cells distended with fat, the nuclei must number at least two In theory, the maximum size these structures could attain would be great It is apparent that two relatively small lipodiastaemata might form a single large one by a process similar to that by which liver cells combine to form new lipodiastaemata By extension of this principle, successive generations of lipodiastaemata could be created of ever increasing magnitude With each new generation, the total number of lipodiastaemata in any lobule would be reduced Diameters of lipodiastaemata greater than 100 μ are rare (Fig 16) Nuclei, counted in serial sections have totalled as many as 80, in a few lipodiastaemata so studied There is the danger however that large nuclei present in successive sections may have been counted twice Plastic reconstructions in three-dimensions should be prepared for greater accuracy

The structure of a lipodiastaema might be compared to that of an acinus in the thyroid gland Both possess a central lumen filled with fat in the one case and colloid in the other The walls of both are formed by a continuous single layer of epithelial cells Unlike the thyroid acinus however the lipodiastaema may have an outlet draining its lumen Bile canaliculi lie between liver cells not separated by sinusoids Dissolution of the septum between two such cells would imply that, in theory at least the lumen of the resultant lipodiastaema could be in continuity with the biliary tree Passages which might appear to drain lumina of lipodiastaemata have not been observed in sections This does not prove their absence however for the walls of lipo diastaemata are so frequently thinned and stretched that small outlets passing through them might not be visualized even if present

The concept of a lipodiastaema as presented here is that of a struc ture If this concept is correct what then is the function of this structure?

It is a mathematical law that, of the total space occupied by a hollow sphere, the percentage of that space taken up by its walls must rapidly diminish as the size of the sphere increases Thus a single large sphere whose volume is equal to the sum of many separate small spheres occupies less space than does the group of small spheres even if the latter are crowded together The fusion of many small unicellular fat vacuoles into multicellular lipodiastaemata each of which contains a single large pool of fat facilitates maximum fat storage in a minimum of space according to the mathematical law just stated The high ratios

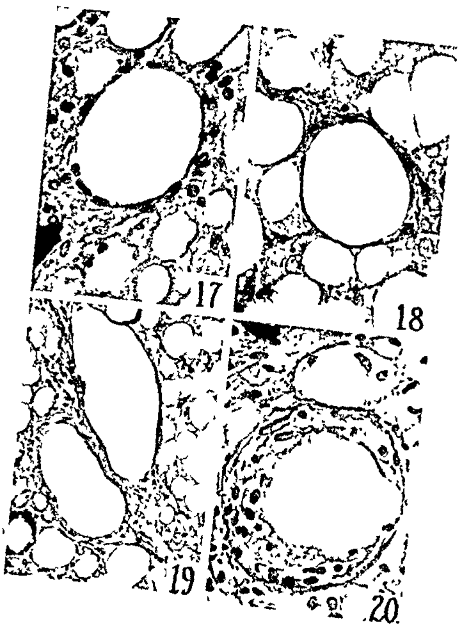
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FIGURE 17 A lipodiastaema with many nuclei present in a single section of its wall This increase in nuclei has probably been produced by shrinkage of the structure rather than proliferation of the cells Liver of a rat choline deficient for 65 days Paraffin section McGregor stain x 860

FIGURE 18 Another atrophic lipodiastaema showing fibrous tissue spreading out to encircle the structure The central vein of the lobule lies just to the left of the field shown The fibrous tissue could be traced to the adventitial sheath of the vein Liver of a rat choline deficient for 65 days Paraffin section McGregor stain x 860

FIGURE 19 Two large lipodiastaemata cut in such a plane that they appear to have a longitudinal axis They are surrounded by a fine skein of connective tissue fibrils Their appearance is not unlike that of abnormal distended bile ducts They are not vessels for the animal was injected with india ink which filled all branches of veins and arteries From a liver of a choline deficient rat (75 days) Paraffin section McGregor stain, x 420

process of shrinkage
The nuclei of some
is surrounded by a
fine band of connective tissue which has an appearance not unlike that of a basement membrane From the liver of a rat choline deficient for 75 days Paraffin section McGregor stain x 860


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of fat to parenchyma that can be produced in the liver would probably *not be possible without this device by which lipodiastaemata are formed* If the fat had always to be stored in relatively small vacuoles, each in individual liver cells, the potential efficiency of the liver as a lipid storehouse might well be much less than it actually is Thus the function of lipodiastaemata is probably to enable the liver to store fat in large amounts

The Fate of Lipodiastaemata in Cirrhosis

In cases of advanced cirrhosis in both man and animals it has been well *established that the fat content of the liver falls to almost normal* values In microsections of these livers, few, if any, lipodiastaemata can be found Apparently th [illegible] the initial phases of cirrhosis only to [illegible] by which they disappear will now be [illegible]

Soon after the first appearance of large lipodiastaemata, the walls of some appear to contain increased numbers of nuclei lying closely together (*Fig 17*) *This* is most frequently noted in the regions around central veins Mitotic figures in the cells of lipodiastaemata have not been observed at any stage The probable explanation of this apparent increase in the number of cells is that having attained their maximum size, the lipodiastaemata begin to shrink As their diameters decrease, the nuclei in their walls would approach one another so that more would be included in random sections of standard thickness (4 μ) than would have been the case before the advent of shrinkage Further evidence of atrophy of lipodiastaemata is afforded by extensions of connective tissue fibres from the adventitial sheaths of the nearby central veins

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FIGURE 21 Two atrophic *lipodiastaemata surrounded by a fine network of* connective tissue fibrils Note that the cells forming their walls are no longer thinned and stretched These might be compared to abnormal forms of bile ducts From the liver of a rat choline deficient for 75 days Paraffin section McGregor stain x 860

FIGURE 22 A *further stage* of atrophy is illustrated by the lipodiastaema in the center of the field The cells in the wall are finely vacuolated and appear to be degenerating Again this might be compared to an abnormal bile duct From the liver of a rat choline deficient for 75 days Paraffin section McGregor stain x 860

FIGURE 23 The collection of cells in the center of the field arranged in a somewhat acinar manner represents the final result of atrophic changes in a lipodiastaema The lumen here is completely absent From the liver of a rat choline deficient for 90 days Paraffin section, McGregor stain x 860

FIGURE 24 With complete atrophy of the lipodiastaemata the cells disappear leaving only hyaline fragments (grey in photograph) between the connective tissue (black) *Paraffin section of liver of a rat, 90 days choline deficient* McGregor stain, x 860


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(Fig 18) This suggests that the space occupied by the lipodiastaema before shrinkage began is being filled by encircling fibrous tissue When cut in the plane of their greatest diameters, these partially fibrosed and atrophic lipodiastaemata not infrequently resemble distended bile ducts (Fig 19) If the plane of section is tangential to the structure, the lumen of an atrophying lipodiastaema appears to be lined by several layers of cells (Fig 20) As the lumen becomes still smaller the walls lose their thinned and stretched appearance (Fig 21) This type of lipo diastaema may, in some instances, very closely resemble atypical bile ducts (Fig 22) In the final stages the lumen disappears altogether (Fig 23) with the result that only a collection of dedifferentiated liver cells is left These cells undergo further degeneration, eventually becom ing only hyaline masses intermingled with fibrous tissue which has increased at the same time (Fig 24) It appears that in the manner the fibrous tissue spreads outward from the central veins preceded by a zone of atrophic lipodiastaemata Eventually all the lipodiastaemata disappear and are replaced by fibrous tissue which then surrounds only nodules of regenerating parenchyma The details of these last stages will be considered in a subsequent section (Figs 25 and 26)

The factors which limit the growth of lipodiastaemata and are re sponsible for their atrophy and disappearance have not been demon strated It is likely, however, that the partial ischaemia present in the centrolobular regions where this type of atrophy occurs may play an important role The loss of fat from those lipodiastaemata which atrophy could be either a cause or a result of their decrease in size The route by which the fat leaves the structures is unknown Absorption into the blood stream or lymph might be possible For this to occur, the fat would have to pass either through or between the epi

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FIGURE 25 This illustrates the growth of connective tissue around atrophic lipodiastaemata The connective tissue fibres are young and have not stained intensely From the liver of a rat, 90 days choline deficient Paraffin section, McGregor stain x 410

FIGURE 26 Degenerating liver cells (derived mostly from lipodiastaemata which have atrophied probably) are intermingled with connective tissue From the liver of a rat 96 days choline deficient Paraffin section, McGregor stain x 410

FIGURE 27 Lipodiastaemata formation around a central vein, low power Those in the centre of the photograph are atrophying and becoming separated from the surrounding liver tissue From the liver of a rat choline deficient for 41 days Frozen section Sudan IV and haematoxylin x 80

FIGURE 28 High power view of a section from the liver shown in Figure 27 above A lipodiastaema lying adjacent to the central vein in the photograph is filled with fat (grey) A lipodiastaema undergoing atrophy is in the upper right portion From the liver of a rat choline deficient for 41 days Frozen section Sudan IV and haematoxylin x 240


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thelial cells forming the walls of lipodiastaemata In such an event it might be expected that a microscopic picture would be produced similar to that observed when fat traverses the epithelium of intestinal villi during fat absorption No suggestion of this has ever been found in frozen sections of livers containing atrophic diastaemata It has been stated that theoretically the lumena of the lipodiastaemata could be in communication with the biliary tree This raises the further possibility that the fat might leak out in the bile E R LeCount and E A Long (J Exp Med *19* 233 (1914)) found the percentage of fat in the bile was increased in fatty degeneration of the liver in man It is planned to investigate this possibility further in choline deficient rats

The Site of Earliest Proliferation of Fibrous Tissue

It has been stated in the foregoing several times that the site of initial proliferation of fibrous tissue in these livers is around the central veins The evidence supporting this will now be presented

Although liver lobules are most frequently cut in a tangential plane which does not include both portal and central areas recognizable in the same section favourable cuts may be found which pass through

a section

There is

unds the

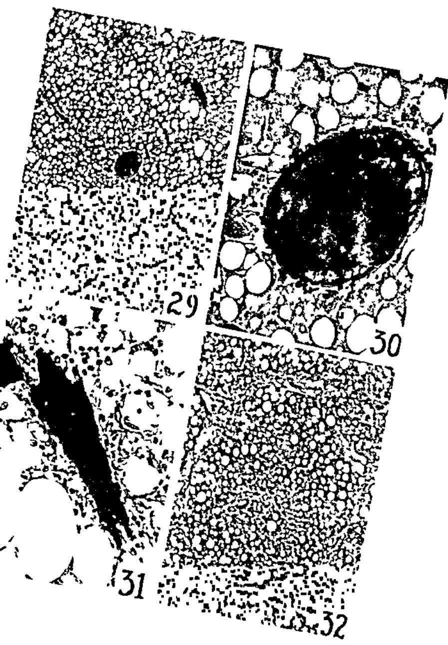
veins shown at the edges of the field included in the photograph The portal region is quite free of fibrosis as shown in the enlargement (Fig 30) This indicates that the areas of early fibrosis are actually centrolobular and surround branches of the hepatic vein (Fig 31) The atrophic lipodiastaemata may bear a superficial resemblance to atypical bile ducts but in frozen sections such structures are filled with stainable fat (Figs 27 and 28)

FIGURE 29 Small crescent shaped areas of fibrous tissue incompletely circle the portal vein in the center of the field The fibrous tissue appears as grey areas in the upper right and lower left portions of the field They surround ink filled veins (black) Liver of a rat choline deficient 56 days Paraffin section McGregor stain x 120

FIGURE 30 This is a high power of the central portion of Figure 29 above It shows that this area of the field (in which no fibrous tissue is present) is the portal region for the large portal vein small hepatic artery and bile duct are clearly shown x 860

FIGURE 31 This is a high power view of the portion of the section shown in the upper right area of Figure 29 It demonstrated early fibrous tissue prolifera tion (slight) around a central vein (black) in the midst of atrophic lipodiastae mata x 860

FIGURE 32 The portal area in the center of the field is completely encircled by connective tissue extending around the central veins From the liver of a rat choline deficient for 79 days Paraffin section McGregor stain x 120


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The fibrous tissue spreads out in the form of trabeculae which connect nearby branches of the hepatic vein one with another This creates an apparent reversal of the usually conceived lobular pattern for the portal canals now lie in the centers of islands of liver tissue which are encircled by the fibrous tissue extending from one central vein to another (Figs 32 and 33) If the plane of section passes through only the portal vein but m sses the smaller bile duct and hepatic artery and if atrophic lipodiastaemata in the centrolobular fibrotic regions are mistaken for bile ducts the encircling pattern of the fibrosis may erroneously be interpreted as portal We suggest that this is the explanation for the contradictory statements in the literature concerning the distribution of these fibrous trabeculae The bile ducts in the fibrotic ares have been stated by some authors to be atypical or poorly formed (C L Connor and I L Chaikoff Proc Soc Biol & Med 39 356 ... 34 1035 (1942)) ... s of fibrosis show ... e have illustrated (Fig 31 and others)

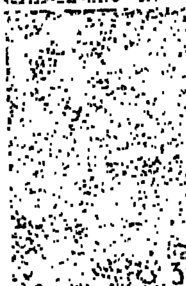
Additional evidence of another type which supports the view that the fibrous tissue is centrolobular was obtained in the following manner It has already been shown that when choline is restored to the d et of choline deficient animals with fatty livers the only cells depleted of stainable lip d within the first 24 to 48 hours are those which surround the portal veins The cells in the centrolobular regions remain fatty for a longer period A special group of animals rats in which hepatic fibrosis had previously been produced by choline deficiency were killed 24 hours and 48 hours after restoring lipotropic factors to their food Frozen sections of these livers showed that the fat had disappeared only from those cells farthest from the fibrous trabeculae

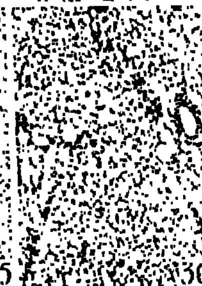
FIGURE 34 L ver of a rat fed the choline defic ent diet for five months The vessels (black) are filled w ... some of the largest l podiastaemata ... s p cture is for compar son w th F g ... IV and haematoxylin x 50

FIGURE 36 Liver of a chol ne deficient rat n wh ch the sheath of the hepatic ve n was injected w th Ind a Ink The black ink is present throughout the strands of grey fibrous tissue McGregor stain Paraffin sect on x 100


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(Figs 34 and 35) The strands of fibrous tissue are surrounded by haloes of parenchymal cells which still contain stainable fat This *strongly suggests that this fibrous tissue is in centrolobular, not peri portal, regions*

Tangential cuts of liver lobules frequently suggest that the fibrosis *is midzonal for the resulting sections may include portions of the* fibrous trabeculae but not the veins which they surround *It can be* demonstrated however, that the fibrous bands are always in continuity with the *adventitial sheaths of the veins This purpose was served by* the preparations made by injections of ink into the sheath of the hepatic vein In the sections the injection mass can be followed from the adventitial

(Fig 36)

that advan

Lillie (Am J Path *23*, 159 (1947)), leaves little doubt that the dis *tribution of fibrous tissue is nonportal throughout the livers of rats in* which dietary cirrhosis has been produced by choline deficiency

Parenchymal Proliferation in Dietary Cirrhosis

Few of the liver cells adjacent to portal canals take part in the formation of lipodiastaemata for here these are rarely found (Figs 29 and 37) *However, it is in these periportal portions of the lobules that* mitotic figures are frequently observed They are not seen in appreciable numbers until the animals have been fed a choline deficient diet for approximately three months or more As this corresponds to the time when fibrosis of *atrophic lipodiastaemata is actively progressing* there is coexistent centrolobular degeneration and portal regeneration in these livers (Figs 37 38 and 39) The young liver cells rarely undergo more

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FIGURE 37 In the center of the field regenerating liver tissue near a portal canal is relatively devoid of fatty change Giant lipodiastaemata surround the fibrous tissue around the central veins which are at the edges of the field Paraffin section McGregor stain x 50 *From the liver of a rat choline-deficient* for 5 months

FIGURE 38 A mitotic figure in a portal area of a liver in the stage of early cirrhosis Paraffin section McGregor stain x 950 (This is from an animal of one of Professor E A Sellers experiments of 1947)

FIGURE 39 A mitotic figure in a liver cell near a portal vein (not shown) This field was Paraffin section

FIGURE 40 A cleared slice (100 μ) of liver tissue injected intravascularly with India Ink Note the small islands of sinusoids widely separated by relatively avascular *bands of fibrous tissue* The liver was *from* a rat fed the choline deficient diet for six months x 50


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than slight fatty change even though the animal is still consuming a choline deficient diet We cannot offer any explanation for this The absence of any appreciable degree of fatty vacuolation in the new tissue serves to differentiate it, in sections from the older parenchyma With completion of atrophy of the lipodiastaemata in the centrolobular regions the fibrous trabeculae surround irregular islands formed almost entirely by regenerating liver tissue which contains little stainable fat On the surface of the organ the elevated nodules of parenchyma are separated by depressions in which run the fibrous bands This produces the appearance which is so characteristic of these livers on gross examination Cleared slices (100 μ) of these organs injected intra vascularly with ink reveal small groups of sinusoids which are widely separated by relatively avascular bands of cleared fibrous tissue (Fig 40)

Ceroid

In 1942 R D Lillie L L Ashburn W H Sebrell F S Daft and J V Lowry (U S Pub Health Rep 57, 502) gave the term *ceroid* to the yellow pigment which is present in the form of large granules and globules in the fibrous trabeculae of cirrhotic livers of choline deficient rats This pigment is insoluble in the usual fat solvents and is accordingly retained in sections prepared with paraffin infiltration This property plus its sudanophilic character renders it unique It also stains with most of the aqueous basic dyes and is acid fast The substance was reported almost simultaneously by R D Lillie F S Daft W H Sebrell (U S Pub Health Rep 57 562 (1941)), H Blumberg and E V McCollum (Science 93 598 (1941)), J E Edwards and J White (J Nat Cancer Inst 2 157 (1941)) and P Gyorgy and H Goldblatt (J Exp Med 75 355 (1942)) Its fluorescent character was described by H Popper P Gyorgy and H Goldblatt (Arch Path 37 161 (1944)) K M Endicott and R D Lillie (Am J Path 20 149 (1944)) reported its staining properties after investigation by a large number of methods and in addition described a few of its chemical characteristics as exhibited in a crude extract K M Endicott (Arch Path 37 496 (1944)) later noted the similarity of ceroid to oxidized unsaturated fat He found that a substance similar to ceroid could be produced in rats by subcutaneous injection of cod liver oil or linseed oil Endicott also found ceroid like pigment in a case of pneumonia in man following aspiration of cod liver oil Similar cases in which acid fast material was present in the lung had been reported earlier by H Pinkerton (Arch Path 5 380 (1928)) and H Graef (Arch Path 28 613 (1939))

K M Endicott F S Daft and W H Sebrell (Proc Soc Exp Biol & Med 57 330 (1944)) succeeded in producing dietary cirrhosis

without ceroid in rats fed diets low in choline a[illegible] of both fat and cod liver oil They felt the latter substance was an important factor in diets used in their previous experiments in which ceroid was found They noted that the ceroid producing diet of P. Gyorgy (Am J Clin Path 14, 67 (1944)) also contained cod liver oil

M Wachstein (Proc Soc Exp Biol & Med 59, 73 (1945)) found that the amount of ceroid in cirrhosis produced in male weanling rats fed on a choline free, protein deficient diet for 90 days could be markedly reduced if the intake of cod liver oil was restricted to one drop weekly In livers of other rats fed the same diet containing 2 percent cod liver oil, large amounts of ceroid were found However, in livers of a few rats fed the same basal diet without any cod liver oil, he found small amounts of ceroid Wachstein concluded, therefore, that although ceroid deposition was not caused solely by ingested cod liver oil it could markedly enhance formation of the pigment

Ceroid was not found in cases of experimental selenium cirrhosis or of human cirrhosis examined by R D Lillie, L L Ashburn, W H Sebrell, F S Daft and J V Lowry (U S Pub Health Rep 57, 502 (1942)) A similar examination of more than 200 normal and pathological human livers gave negative results (H Popper, P Gyorgy and H Goldblatt, Arch Path 37, 161 (1944))

J Victor and A M Pappenheimer (J Exp Med 82 375 (1945)) called attention to the resemblance of ceroid to the acid fast pigment found in the uterine muscle and adipose tissue of rats fed diets deficient in vitamin E They found that the food mixtures reported in the literature to have produced large amounts of ceroid in dietary cirrhosis were deficient in this vitamin They performed experiments which showed that the production of ceroid in the livers of rats fed on a low protein diet, with or without the addition of excess 1-cystine (5 percent), was transiently inhibited by the administration of alpha tocopherol Cod liver oil (5 percent) in the diet could not prevent this effect of tocopherol They also found that 1 percent choline chloride had an inhibiting effect on the deposition of liver-ceroid resulting from a low protein containing excess cystine The authors do not discuss this finding In our opinion, these experiments are difficult to interpret as the levels of the supplements (cystine choline) are rather high

A M Pappenheimer and J Victor (Am J Path 22 395 (1946)) examined tissues from human autopsies and reported the presence of acid fast pigment not only in livers, but also in many other organs These findings which do not agree with earlier examinations of human tissue (Lillie Ashburn Sebrell Daft and Lowry, *loc cit* Popper, Gyorgy and Goldblatt *loc cit*) may be open to question as the authors do not state if the pigment they found was sudanophilic and fluorescent

than slight fatty change even though the animal is still consuming a choline deficient diet We cannot offer any explanation for this The absence of any appreciable degree of fatty vacuolation in the new tissue serves to differentiate it, in sections, from the older parenchyma With completion of atrophy of the lipodiastaemata in the centrolobular re gions, the fibrous trabeculae surround irregular islands formed almost entirely by regenerating liver tissue which contains little stainable fat On the surface of the organ, the elevated nodules of parenchyma are separated by depressions in which run the fibrous bands This produces the appearance which is so characteristic of these livers on gross examination Cleared slices (100 μ) of these organs injected intra vascularly with ink reveal small groups of sinusoids which are widely separated by relatively avascular bands of cleared fibrous tissue (Fig 40)

Ceroid

In 1942 R D Lillie, L L Ashburn W H Sebrell, F S Daft and J V Lowry (U S Pub Health Rep *57*, 502) gave the term *ceroid* to
t [illegible] n the form of large gran les and
g [illegible]
r [illegible]
ingly retained in sections prepared with paraffin infiltration This property plus its sudanophilic character renders it unique It also stains with most of the aqueous basic dyes and is acid fast The substance was *reported almost simultaneously by* R D Lillie, F S. Daft, W H Sebrell (U S Pub Health Rep 57, 562 (1941)), H Blumberg and E V McCollum (Science *93*, 598 (1941)), J E Edwards and J White (J Nat Cancer Inst 2 157 (1941)), and P Gyorgy and H Goldblatt (J Exp Med *75* 355 (1942)) Its fluorescent character was described by H Popper P Gyorgy and H Goldblatt (Arch Path *37* 161 (1944)) K M Endicott and R D Lillie (Am J Path *20, 149* (1944)) reported its staining properties after investigation by a large number of methods and in addition described a few of its chemical characteristics as exhibited in a crude extract K M Endicott (Arch Path *37* 496 (1944)) later noted the similarity of ceroid to oxidized unsaturated fat He found that a substance similar to ceroid could be produced in rats by subcutaneous injection of cod liver oil or linseed oil Endicott also found ceroid like pigment in a case of pneumonia in man following aspiration of cod liver oil Similar cases, in which acid fast material was present in the lung had been reported earlier by H Pinkerton (Arch Path *5* 380 (1928)) and H Graef (Arch Path *28*, 613 (1939))

K M Endicott F S Daft and W H Sebrell (Proc Soc Exp Biol & Med *57* 330 (1944)) succeeded in producing dietary cirrhosis

without ceroid in rats fed diets low in choline and free of both fat and cod liver oil They felt the latter substance was an important factor in diets used in their previous experiments in which ceroid was found They noted that the ceroid producing diet of P Gyorgy (Am J Clin Path 14, 67 (1944)) also contained cod liver oil

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as well as acid fast They pointed out that the administration of large amounts of cod liver oil is known to favor destruction of vitamin E They concluded that the accumulating evidence suggesting a relation between vitamin E deficiency and ceroid, although strong, needed further investigation

P Gyorgy and H Goldblatt recently (J Exp Med *89*, 245 (1949)) reported that tocopherol, even when given in very large doses (30 mgm daily), will not prevent the formation of ceroid when cod liver oil is ingested and will reduce only slightly the total quantity found in the cirrhotic livers of choline deficient rats They were able, however, to produce dietary cirrhosis without ceroid provided that cod liver oil, and to a lesser degree other sources of unsaturated fatty acids, were eliminated from the experimental diet

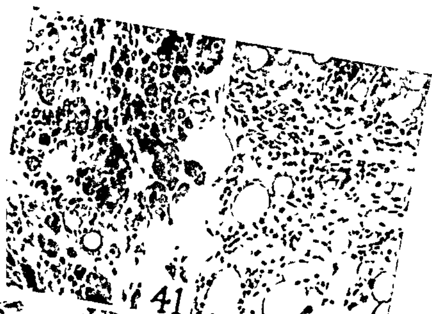
Masses of ceroid were present in the fibrous trabeculae in the livers of those animals in our experiments in which the basal diet alone was fed (Fig 41) This diet is deficient in tocopherol, and although the mixture does not contain the crude form of cod liver oil, it is present in the form of a concentrate In a group of ten animals fed the same basal diet with a supplement of tocopherol little or no ceroid could be demonstrated when they were sacrificed at the end of five months (Fig 42) These results appear to support the conclusions of A M Pappenheimer and J Victor (Am J Path *22*, 395 (1946)) The apparent discrepancy between our observations and those of P Gyorgy and H Goldblatt (J Exp Med *89*, 245 (1949)), concerning animals given supplements of tocopherol and cod liver oil may possibly be due

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FIGURE 41 Sudanophilic masses of ceroid appear as dark grey masses in the fibrous tissue in which a few atrophic lipodiastaemata appear From the liver of a rat fed the basal, choline deficient diet for five months Paraffin section, Sudan IV and haematoxylin stain, x 500

FIG[illegible] the liver of the [illegible]
rat [illegible]
to a [illegible]
to the rat whose liver is represented in this figure Paraffin section Sudan IV and haematoxylin x 500

FIGURE 43 Fibrils of connective tissue are shown extending around and almost [illegible] degenerating liver cell containing small amounts of ceroid in the form [illegible]
with red blood cells [illegible]
[illegible]
x 860


41
42


43

to the difference in the form in which the latter substance was given, they used the crude product, whereas we employed the concentrate

We have examined the livers of 40 cases of human cirrhosis[5] for the presence of ceroid Paraffin sections were stained with Sudan IV and by the Ziehl Neelson method for demonstrating acid fast material Little ceroid could be found in any of these livers This finding is in agreement with the results of similar surveys by Lillie, Ashburn *et al* (*loc cit*) and Popper *et al* (*loc cit*) but at variance with the report of Pappenheimer and Victor (*loc cit*)

Finally, one or two observations were made concerning the formation of ceroid in the experimental animals The pigment first appeared in the form of small globules in the cytoplasm of regressing cells in walls of atrophic lipodiastaemata (Figs 43 and 44) When these cells have degenerated and been replaced by fibrous tissue, the pigment remains as clumped aggregates scattered through the fibrotic areas (Fig 41) Some of the pigment granules are taken up by macrophages

Summary

1 A progressive series of changes, as followed by microsections of the fatty livers of choline deficient rats which were sacrificed at frequent intervals during the development of typical cirrhotic lesions, has been described An attempt has been made to interpret these changes in terms of the pathogenesis of this type of cirrhosis

2 Small droplets of stainable fat appeared in the centrolobular cells of livers of rats killed after they had access to the basal choline deficient diet for only one day

After seven to ten days, fat was demonstrable in every parenchymal cell During this period the small droplets initially present fused to form single large vacuoles that distended the cells in which they were contained

3 Adjacent portions of limiting membranes of neighboring cells became transformed into tenuous septa by a process of compression and stretching which accompanied the progressive enlargement of the intracellular fat vacuoles separated by the septa In the livers of animals sacrificed after several weeks of dietary choline deficiency, microscopic evidence indicated that some of these thin septa had ruptured A number of septal breaks had the result of combining several liver cells into relatively enormous structures of a multicellular nature, these have been named *lipodiastaemata* These are sacs with unilocular lumina distended with fat The walls of the sacs consist of a continuous single layer of dedifferentiated liver cells The concept, formation,

[5] This material was made available to the writer by kind permission of Professor William Boyd Department of Pathology and Bacteriology University of Toronto

Shorr I wonder, Dr Hartroft, whether your problem might not be helped by working with living rather than dead liver cells Some time ago, when I was interested in the differences in the behavior of liver slices and homogenates, I found that when liver was put through the Latapie mincer, a very high percentage of individual viable liver cells were obtained That they are little injured in the process was evident from their very steady oxygen consumption for several hours (Cold Spring Harbor Symposia on Quantitative Biology, 7, 323, (1939)) With this technic you should be able to study the individual living liver cell, see the distribution of fat droplets and determine how large a lipodiastaema can be and still be contained within a single cell, or whether they are multinucleated giant cells

Hartroft There is no doubt there are many nuclei in the walls of the lipodiastaemata because serial sections very definitely demonstrate that I have counted them The counts have gone up as high as 80 There is danger that I have counted some of them twice where portions appeared in neighboring sections The fact that lipodiastaemata measure very frequently 100 micra in stained sections and infrequently even higher, suggests I think, that in the fresh state they are larger because of shrinkage in the stained sections

Watson Do you think it is the pressure of the lipodiastaemata that induces the beginning fibrosis or do you have any concept of the exact mechanism of origin of the fibrosis?

Hartroft As to the origin of the fibrosis that may be demonstrated not only by using the injection of ink into the sheath of the veins but also by following the fibrous tissue through the sections In this manner we can demonstrate continuity between fibrous trabeculae, even in the earliest stages with the adventitial sheaths of the central veins In the oil immersion photomicrograph which I showed (Fig 18), the fibrous strands extend around the cell Why lipodiastaemata atrophy I do not know I might suggest that circulation possibly plays a part

Watson Have you stained any sections with Weigert s stain?

Hartroft Yes

Watson Does it show anything unusual?

Hartroft No

Watson Perhaps you are acquainted with that paper of Urteaga Ballon (Arch Rev de Patologia y Clin 2, 171 (1948))? Have you seen his study on the use of Weigert s stain in human livers? He makes the claim that the so called fibrosis of the alcoholic fatty liver is not connective tissue at all but largely elastic tissue emanating from the walls of veins and that one can differentiate these cirrhoses strictly on the basis of Weigert s stain from those in which there is true fibrosis

. ' That is not confirmed in the rat in my experience

Watson I have not found anyone who has repeated Urteaga s study He believes it necessary to use Weigert s stain on fresh material and that old fixed tissue is of little or no value

Goldblatt I have not done a Weigert stain on fresh liver tissue in fatty cirrhosis so cannot comment On the matter of the site of origin of the connective tissue I must admit that I along with all others who first succeeded in producing experimental cirrhosis was guilty of an error which we have all retracted viva voce et verbo scripto We all considered the cirrhosis as primarily portal but we all agree now that originates around the central veins

Since the work of Ashburn there has been no doubt that it is primarily a central type of cirrhosis So as far as I am concerned I am completely in agreement with everything that Dr Hartroft has shown and everything that he has said But there are some things that I would like to ask him and then perhaps we can get things clarified

One is from what he has said it is not quite clear to me whether he thinks there is a definite proliferation of connective tissue an absolute increase of collagenous material and where it comes from and even perhaps if he has some ideas why it originates? What is the stimulus? At no time in his report has he mentioned the word necrosis and that makes me wonder whether I am guilty of another error There is very little doubt that as a result of a diet which as far as I know is exactly the same as that which induces the changes which Dr Hartroft mentioned we have been able to see necrotizing changes in the liver

I see in practically all the livers which show fibrosis remnants of that I interpret as necrotic or extremely degenerated liver cells parenchymal cells Inasmuch as Dr Hartroft did not even mention the word necrotic as applied to liver cells I wonder if he does not subscribe to the idea that there are necrotic liver cells no matter what their cause Whether they are the result of the fibrosis or help to induce the fibrosis that I realize is difficult to settle but I would like to know from him whether he has observed such necrotic cells I have been referring to those as residual necrotic liver cells in my reports to Dr Gyorgy without implying that they are remnants of originally necrotic cells or just ensnared liver cells that have become necrotic I am inclined to the former view for the simple reason that I often see single ensnared normal liver cells surrounded by a mass of thick fibrous connective tissue

Has Dr Hartroft observed such a thing as necrotic liver cells in these livers which he has examined?

One other thing and that is what do you think is the site of formation and deposit of ceroid? Do you find it in Kupffer cells? Do you

think a lot of it that appears to be within cells is actually in Kup cells, or do you think that those cells are altered liver cells?

Gyorgy Before Dr Hartroft answers, may I just add a few rema because they are pertinent It is true that at the meeting of the Endoc Study Section of the U S P H S in Cleveland two years ago I menti that the cirrhotic changes in experimental dietary cirrhosis are not *not portal but also not strictly central What I had in mind wa* differentiate between the purely central cardiac cirrhosis and the n stellate dietary cirrhosis originating around the central vein If die cirrhosis would be caused by anoxia as cardiac cirrhosis it shoulc strictly central

Dr Hartroft stated en passant that in the presence of tocoph in the experimental diet one cannot expect production of ceroid conclusion is not in accord with our own observations Tocopherol not prevent the development of ceroid provided the experime cirrhosis producing diet contains cod liver oil or any unsaturated f acid as fat supplement Even in daily doses of 30 mg, tocopherol only slightly delay but not suppress the production of ceroid

Cellular necrotic foci scattered over the lobules and enmeshed in bands of connective tissue have been called by us 'residual, m recently cellular focal necrosis

Hartroft First, Professor Goldblatt, you asked is there a defi proliferation, an increase in collagen and what is the probable stimul The nicest evidence I think that one could get if there were a defi increase in fibroblasts and collagen, would be to see mitotic figures the fibrous tissue cells I have never seen those On the other hand feel that there is a definite increase in the amount of fibrous tis because the appearance of the section gives everything that sugg that However, Professor Boyd s chief assistant, Dr Barry, from E land, who has been with us one or two years, made the rather ingeni suggestion that he thought it was the reticulum and supporting tis around these cells shoved to the outside of the lipodiastaemata, t gave rise to the deposition of fibrous trabeculae Nevertheless, I f just from the appearance of the sections that there must be a defin increase in the amount of fibrous tissue I certainly think the wc which you mentioned on the estimation supports that Until there evidence to the contrary I probably will continue to believe there is definite increase in collagen and fibrous tissue

समुद्रइव गम्भीर नैव शक्यं चिकित्सितम् ।
वक्तुं निर विशेषेण श्लोकानामयुतैरपि ॥'

—सुश्रुत संहिता

'The Science of Medicine is fathomless like the sea and can not be exhaustively narrated in thousands of couplets'

—SUSHRUT SAMHITA